NIAID/NIH

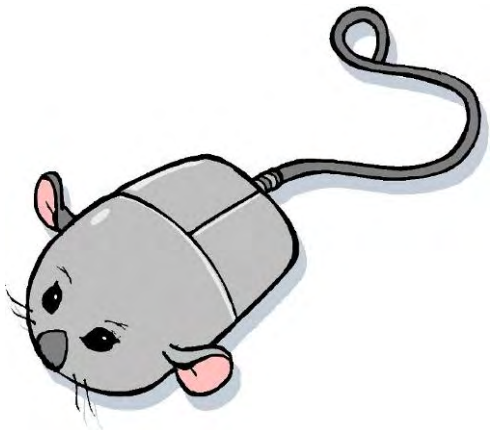
USAMRIID

Nonhuman Primates as a Model for Filovirus Infection

Dr. Tom Geisbert

11 September 2007

Animal Models of Ebola and Marburg Viruses



Mice

- ZEBOV (i.p. route only)
- MARV???

Nonhuman Primates (NHP)

- Rhesus monkeys (ZEBOV, MARV's)
- Cynomolgus monkeys (ZEBOV, SEBOV, REBOV, MARV's)
- African green monkeys (ZEBOV, MARV's)
- Hamadryas baboons (ZEBOV)

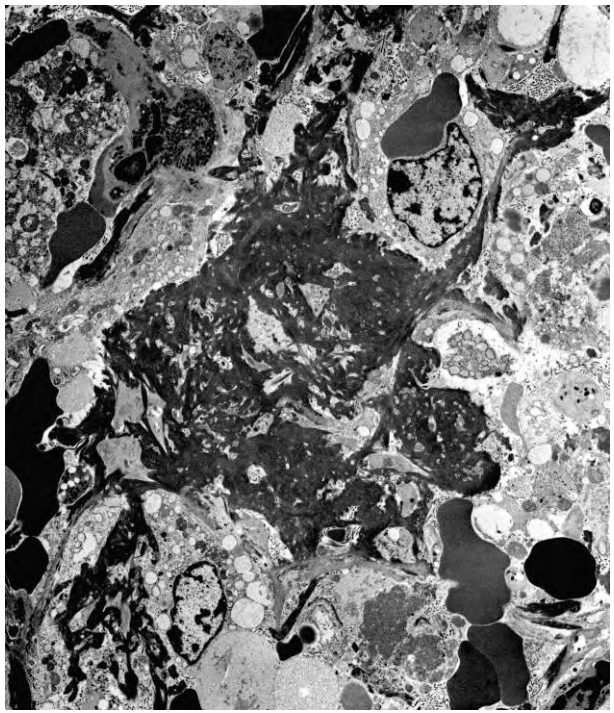
Guinea pigs
(Strain 13, Outbred)

- ZEBOV
- SEBOV?
- MARV-Musoke
- MARV-Ravn
- MARV –'67

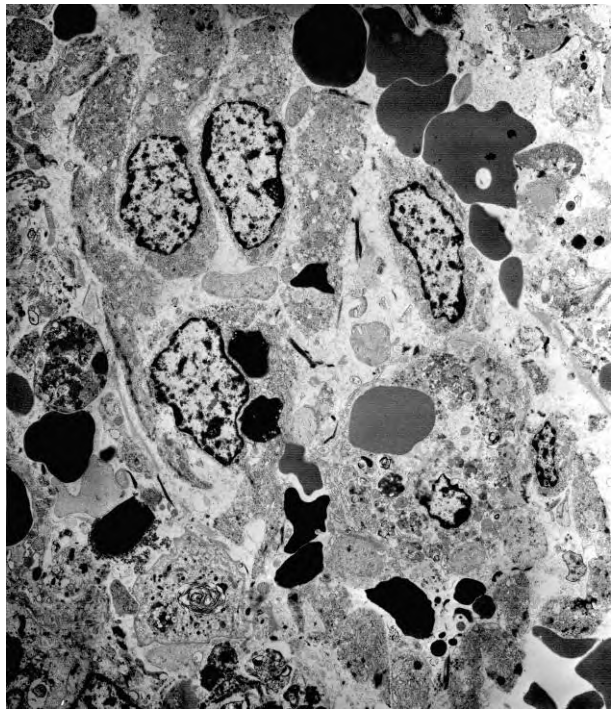
Filovirus – Postexposure Treatments

Compound / Drug	Mouse	G.Pig	NHP	Human
Ribavirin	NT	No	No	NT
S-adenosylhomocysteine	Yes	NT	No	NT
rIFN-alpha	Yes	No	No	NT
rIFN-beta	Yes	NT	No	NT
Equine IgG	Partial	Yes	No	?
Convalescent blood	NT	NT	?	7/8
rHuman monoclonal ab	NT	Yes	0/4	NT
Antisense	Yes	Yes	NT	NT
siRNA	NT	Yes	NT	NT
rNAPc2	NT	NT	Partial	NT
rhAPC (Xigris)	NT	NT	2/11	NT
VSV vaccine	NT	NT	5/5 (4/8)	NT

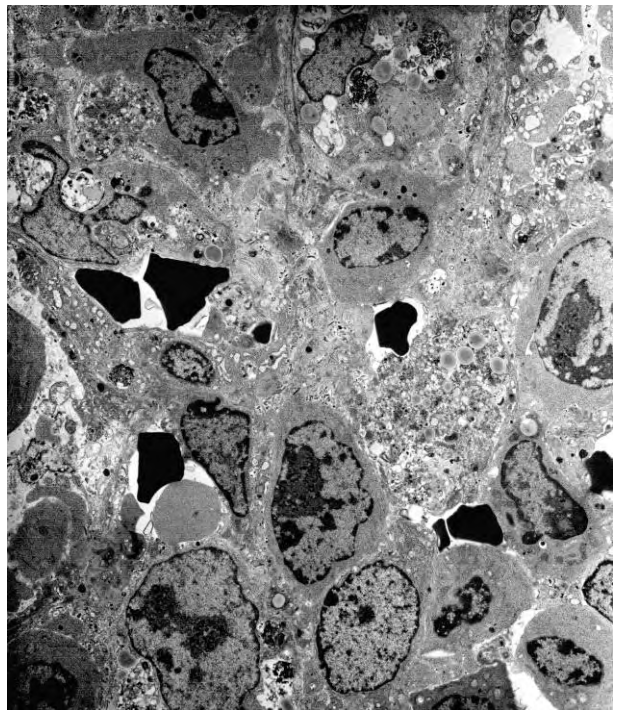
Fibrin Deposition in Spleen of Ebola-Infected Animals



Cynomolgus macaque



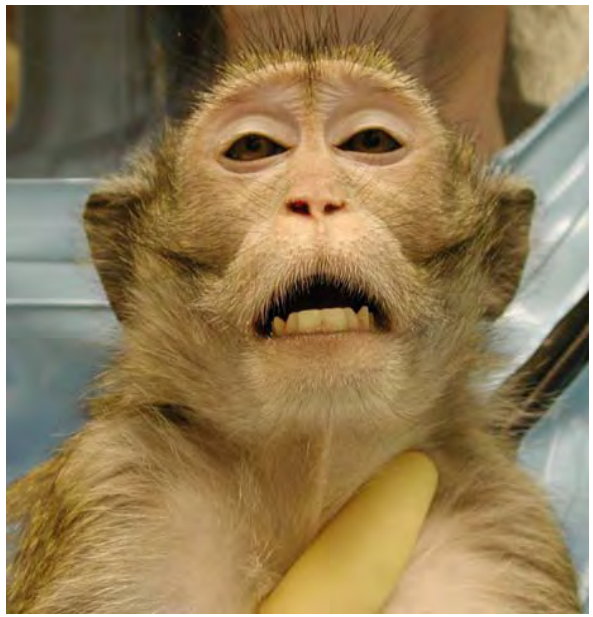
Guinea pig



Mouse

NHP Species Used for Filovirus Studies

Rhesus



Cynomolgus

African Green Monkey

Cost (Primate Products, Inc):

African green monkey: $2,500

Cynomolgus macaque: $4,000

Rhesus macaque (Chinese origin): $4,500

Rhesus Macaques

Subspecies

- Indian Origin
- Chinese Origin

Human-Rhesus Macaque sequence identity is ~ 93%

Cynomolgus Macaques

Philippines, Vietnam, China, Indonesia

Mauritius

Krebs et al. (J Immunol 2005) defined 66 MHC I alleles in cynomolgus macaques of Chinese, Vietnamese, and Mauritian origin. Most MHC I alleles were found only in animals from a single geographic origin, suggesting that cynomolgus macaques from different origins are not interchangeable in studies of cellular immunity

African Green Monkeys

Lack of Macular Rashes

- Marburg (Popp), i.p. (Simpson, 1969)
- Marburg (Popp), s.c. (Haas et al. 1971)
- Ebola-Zaire (’76), i.p. (Bowen et al., 1978)
- Ebola-Zaire (’76), i.p. (Davis et al., 1997)
- Ebola-Zaire (’76), s.c., (Ryabchikova et al., 1999)
- Ebola-Zaire (’76), i.m. (Rassadkin et al. 2000)

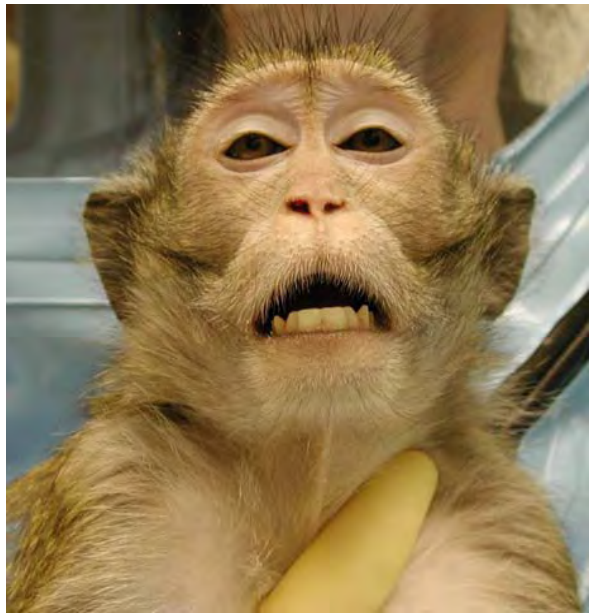
NHP Species Used for Filovirus Studies

Rhesus

Pathogenesis: 22

Vaccine: 2

Treatment: 8



Cynomolgus

Pathogenesis: 10

Vaccine: 8

Treatment: 2

African Green Monkey

Pathogenesis: 11

Vaccine: 0

Treatment: 0

Disease Course in Filovirus-Infected NHP

1000 pfu, i.m. injection

Filovirus	Species	Number	Mortality	Mean Day of Death
Ebola-Zaire (’95)	Cynomolgus	36	100%	6.6 (Mode = 6) (range 5-9)
Ebola-Zaire (’95)	Rhesus	25	100%	8.4 (Mode = 8) (range 7-10)
Ebola-Sudan (Gulu)	Cynomolgus	4	50%	8.5 (7, 10)
Ebola-Sudan (’76)	Cynomolgus	5	100%	7.6 (Mode = 8)
Ebola-Sudan (’76)	Rhesus	1	100%	17
Marburg (Angola)	Cynomolgus	1	100%	8
Marburg (Angola)	Rhesus	6	100%	7.3 (Mode = 7)
Marburg (Ci67)	Cynomolgus	4	100%	7.8 (Mode = 8)
Marburg (Musoke)	Cynomolgus	4	100%	9 (Mode = 9)
Marburg (Musoke)	Rhesus	4	100%	11.3 (Mode = 11)
Marburg (Ravn)	Cynomolgus	1	100%	8

Route of Exposure vs. Disease Course

1976 Outbreak of Ebola-Zaire

Injection

Mean incubation period: 6.3 days

Mortality: 100% (85/85)

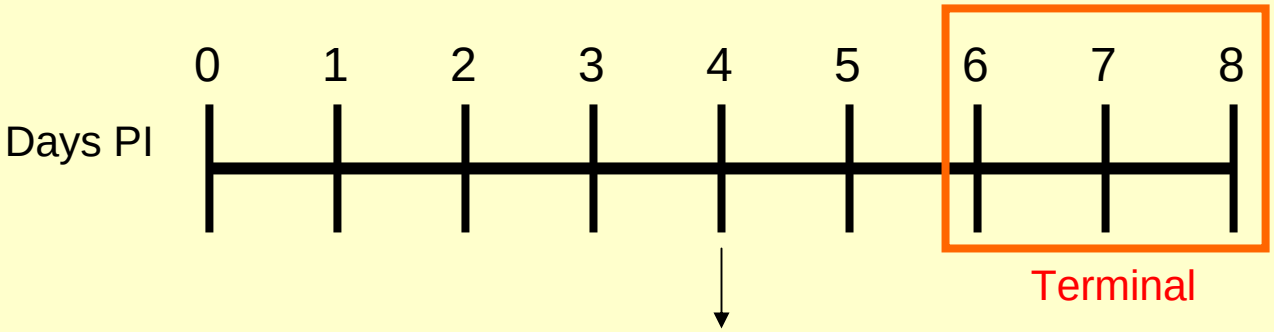
Contact Exposure

Mean incubation period: 9.5 days

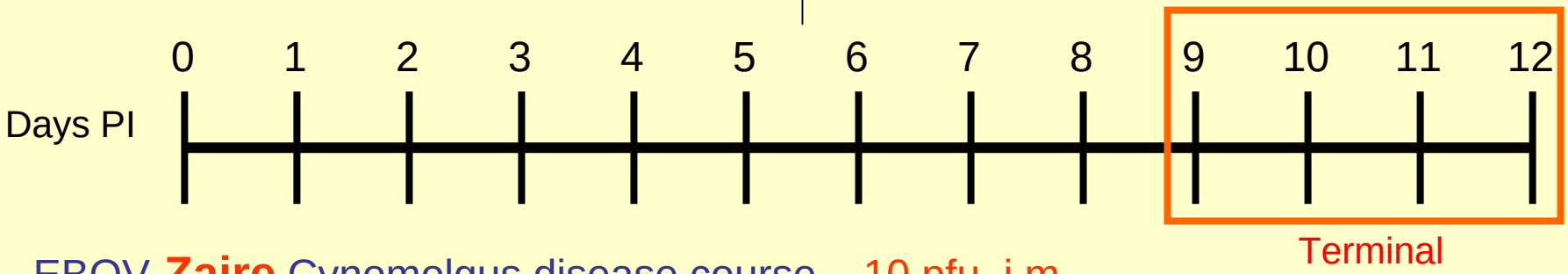
Mortality: 80% (119/149)

Challenge Dose vs. Disease Course

EBOV-**Zaire** Cynomolgus disease course – 1000 pfu, i.m.



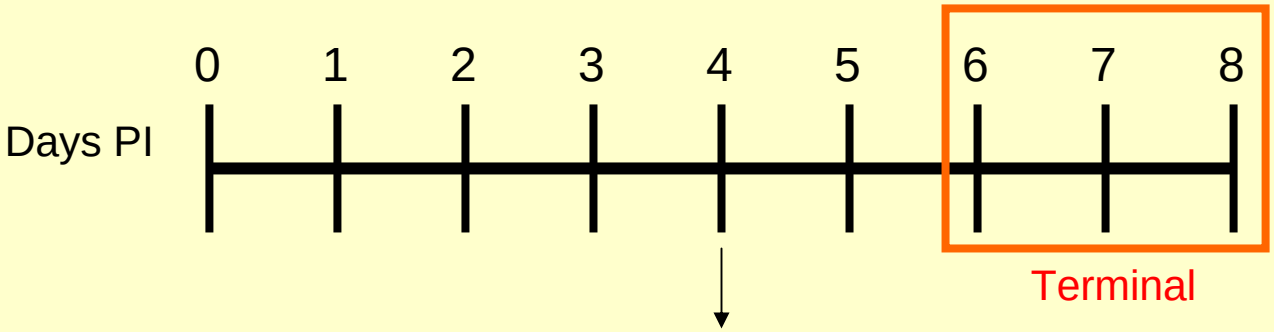
Fever, Viremia,
Rash, Anorexia



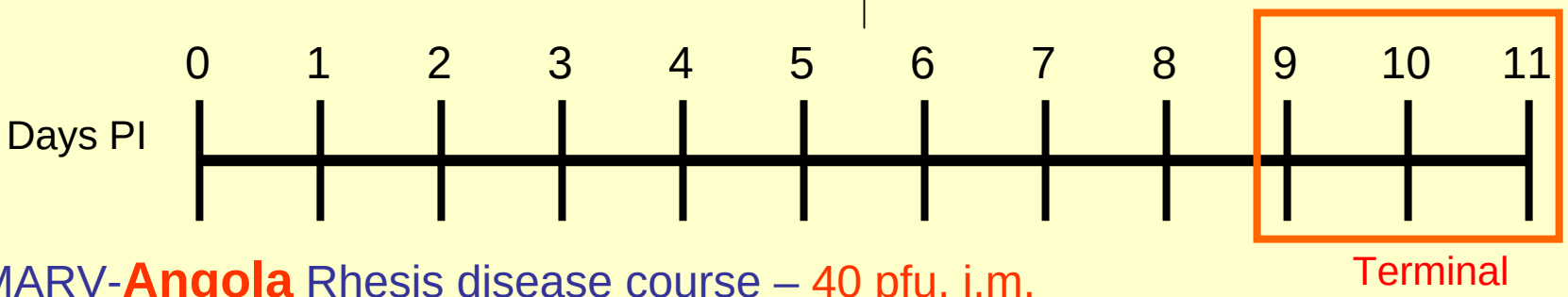
EBOV-**Zaire** Cynomolgus disease course – 10 pfu, i.m.

Challenge Dose vs. Disease Course

MARV-**Angola** Rhesus disease course – 1000 pfu, i.m.



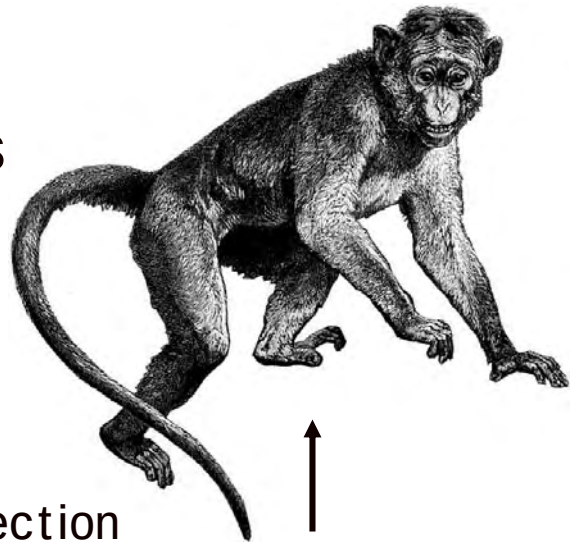
Fever, Viremia,
Rash, Anorexia



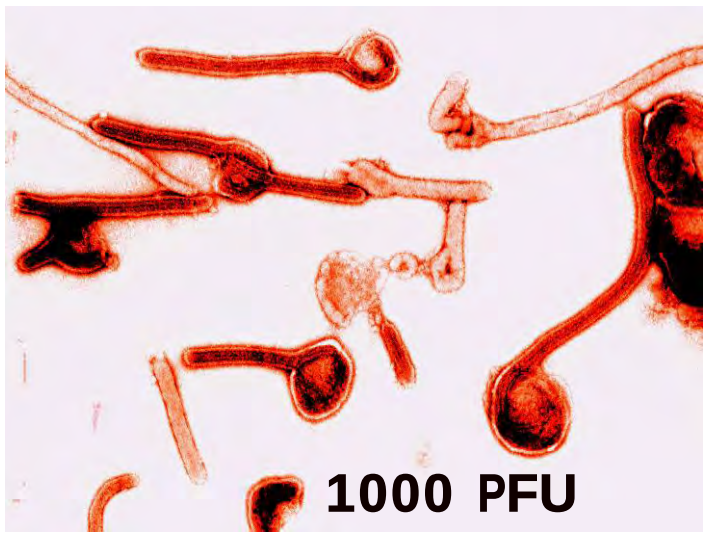
MARV-**Angola** Rhesis disease course – 40 pfu, i.m.

Ebola Pathogenesis Study Design

21 Cynomolgus macaques



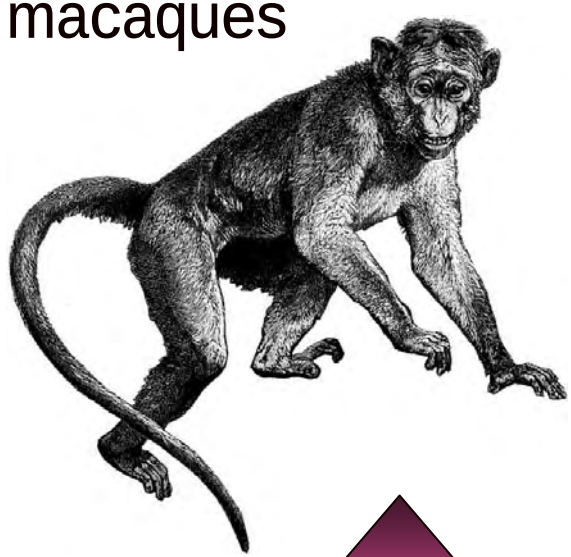
i.m. injection

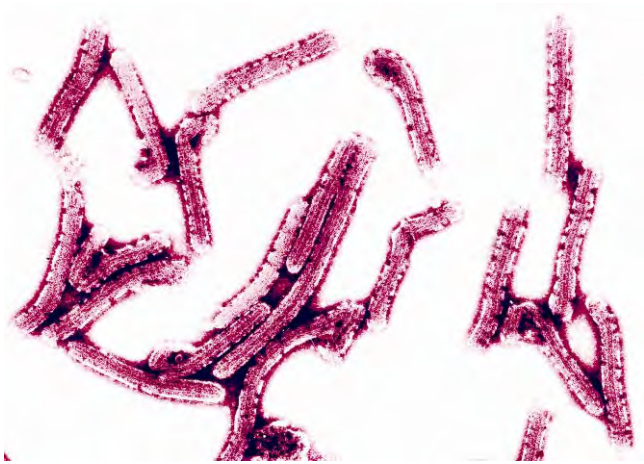


3	d 1
3	d 2
4	d 3
4	d 4
4	d 5
3	d 6

Marburg Pathogenesis Study Design

18 Cynomolgus macaques



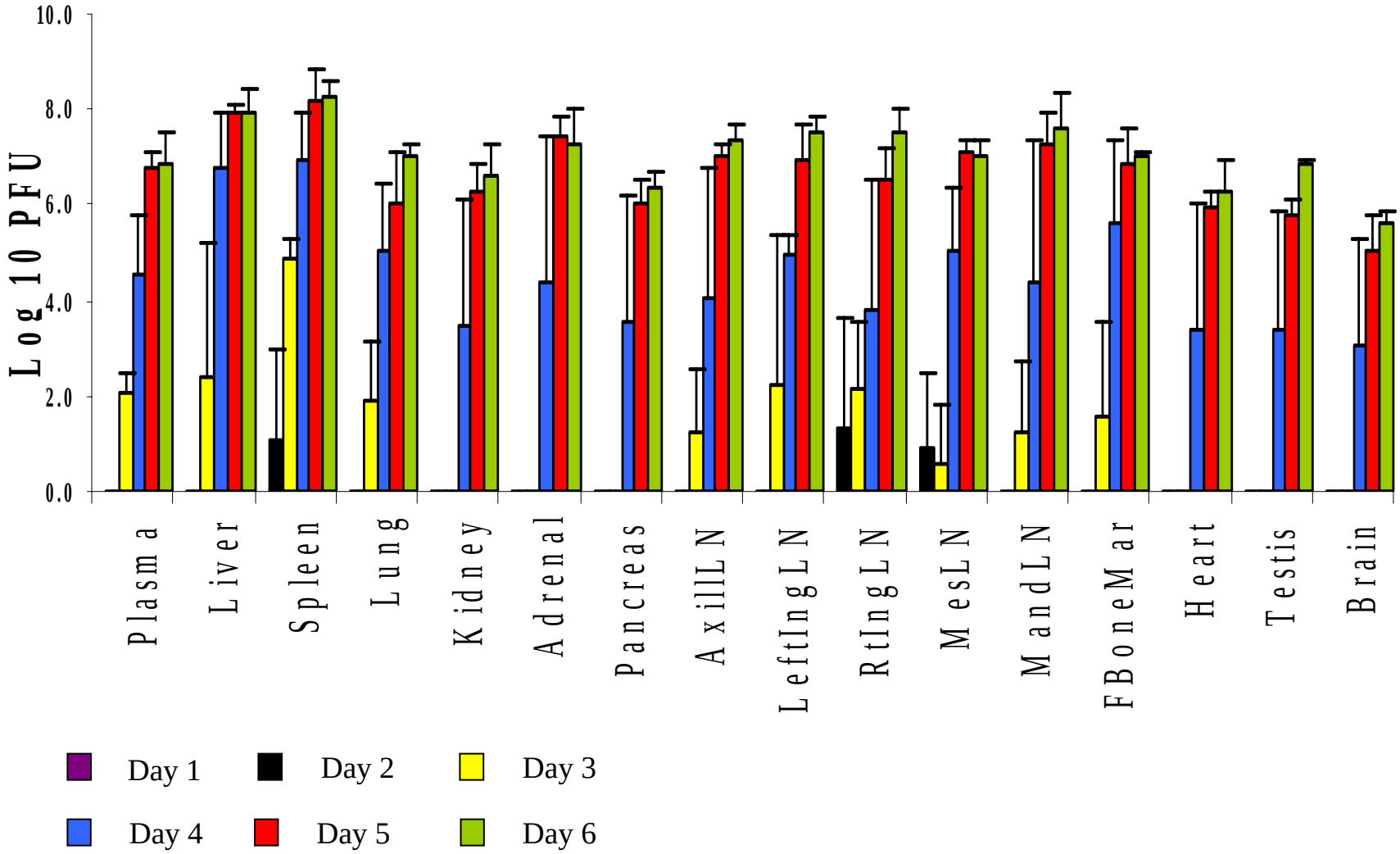


1000 PFU MARV (Ci67)

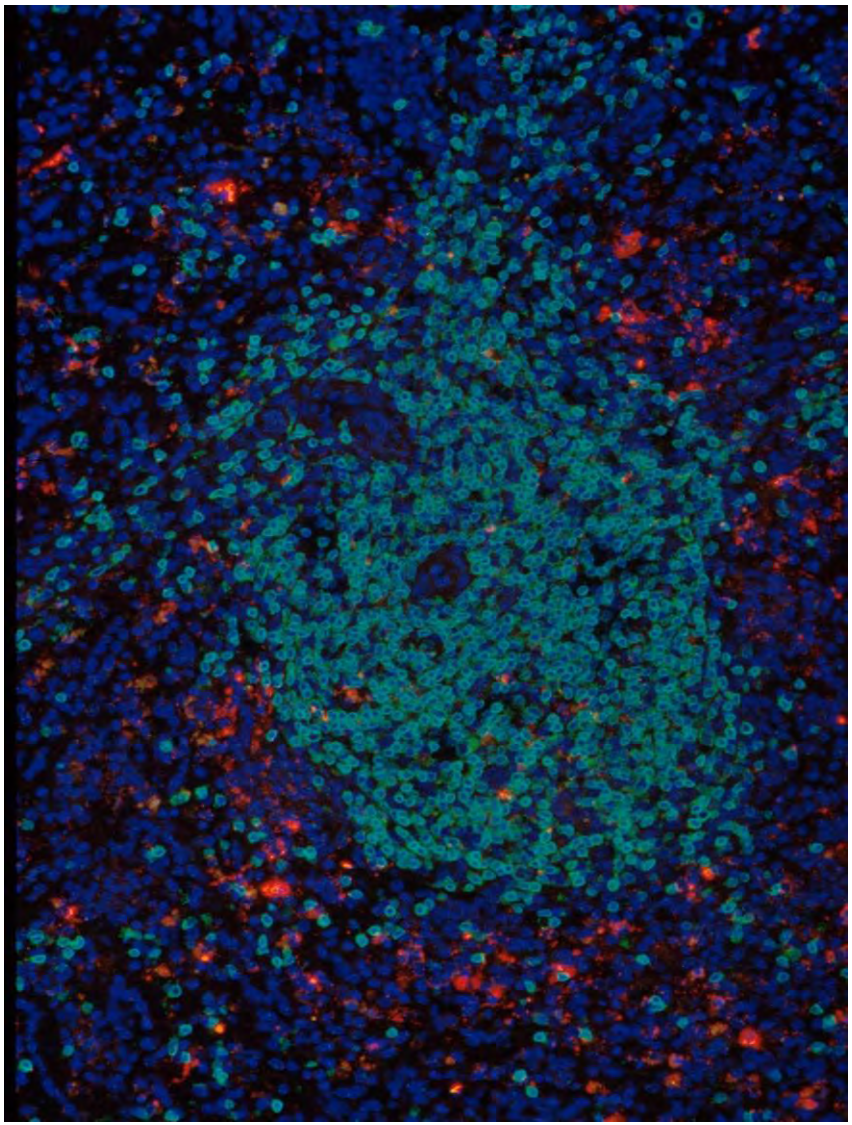
i.m. injection

3	d 2
3	d 3
3	d 4
3	d 6
3	d 7
3	d 8

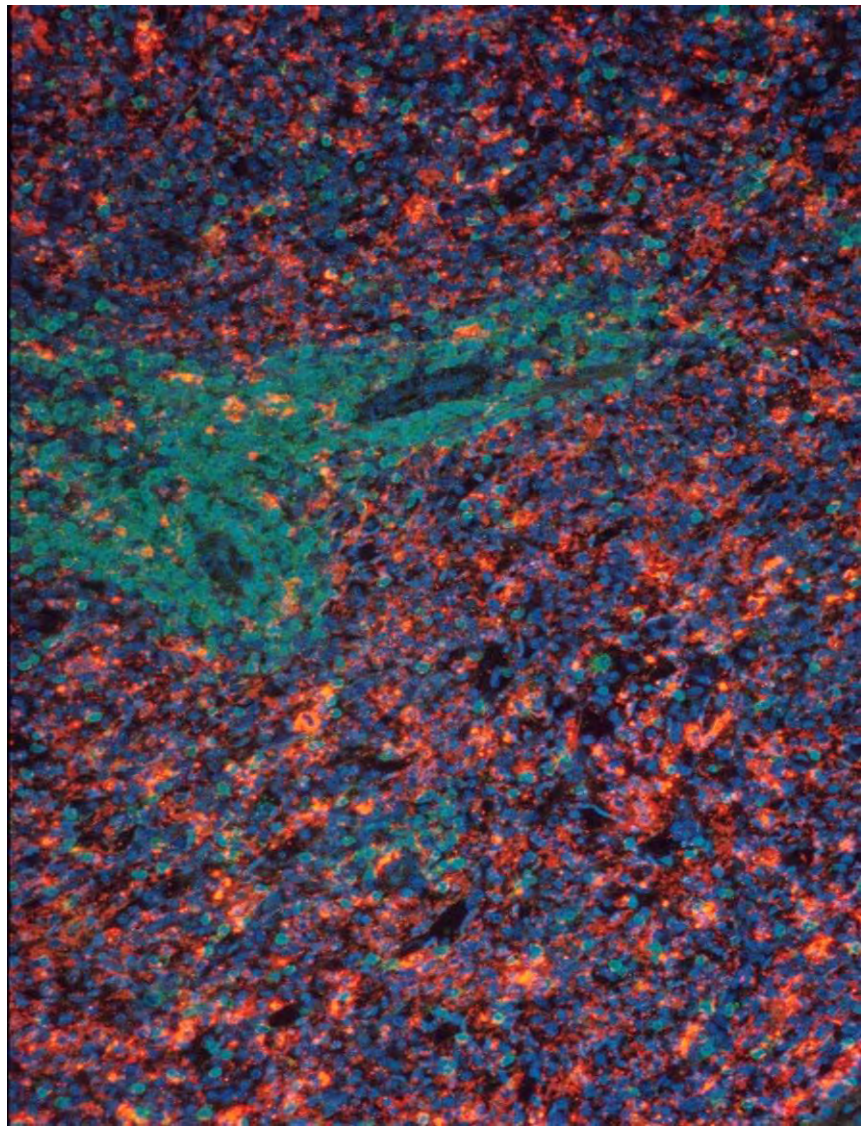
Organ Infectivity Titers - Ebola



Ebola Antigen in Spleen

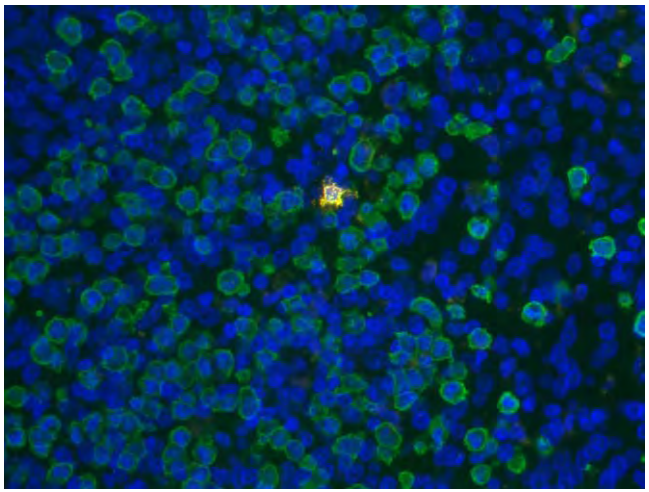


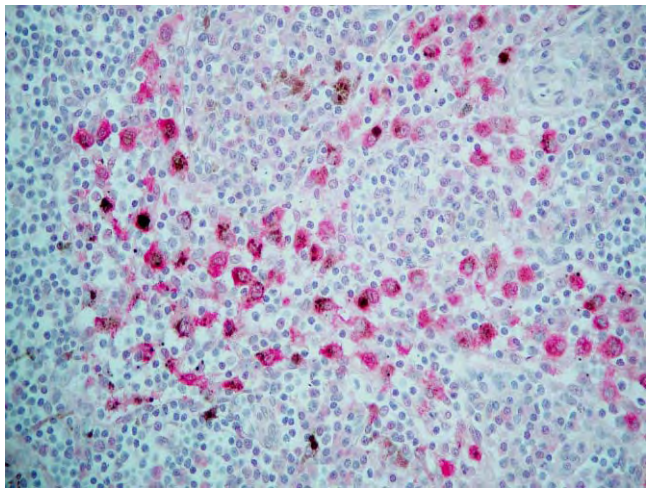
Day 4

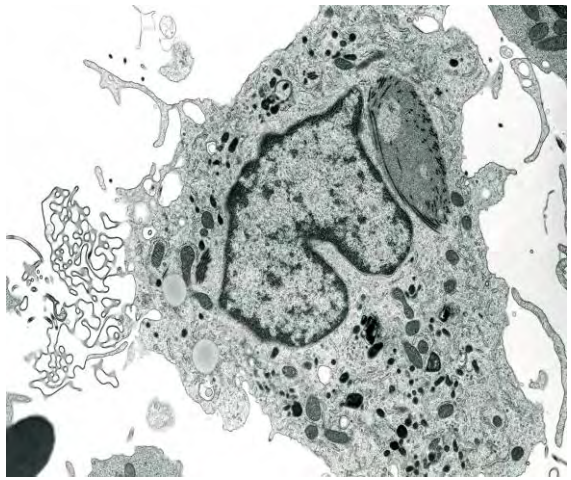


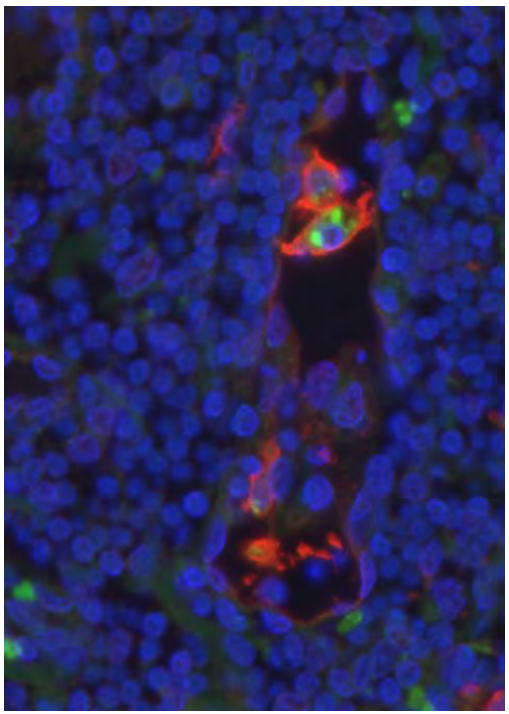
Day 5

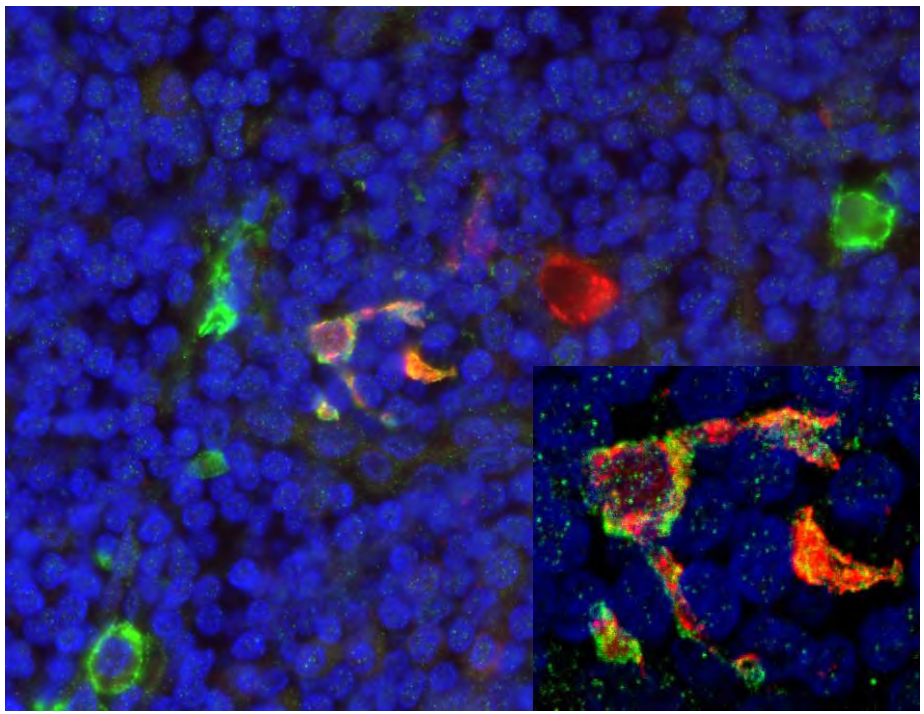
Filovirus Infection of Monocytes/Macrophages and Dendritic Cells

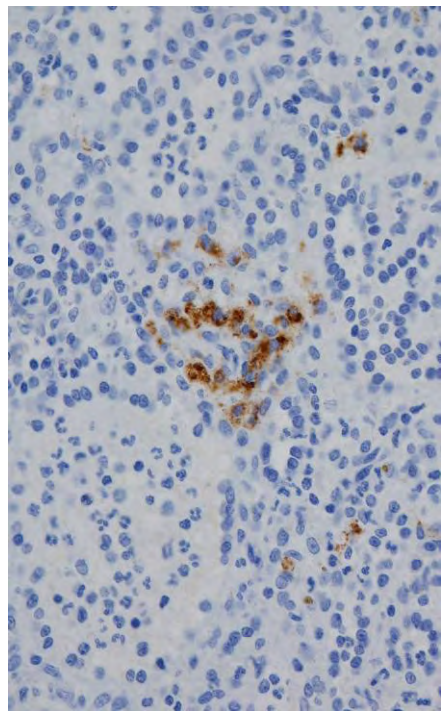




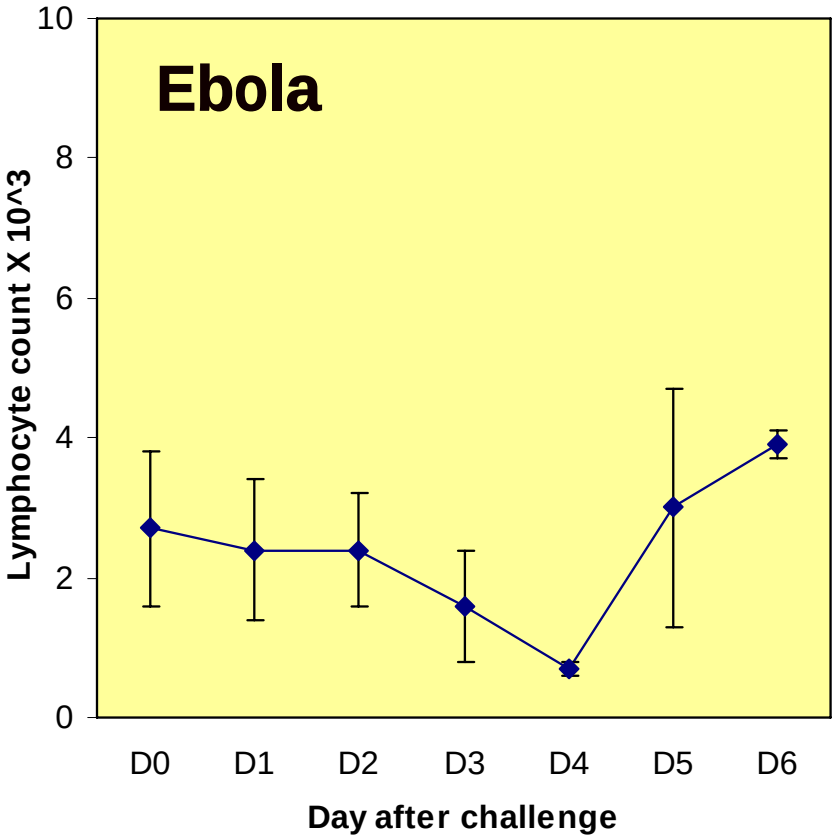


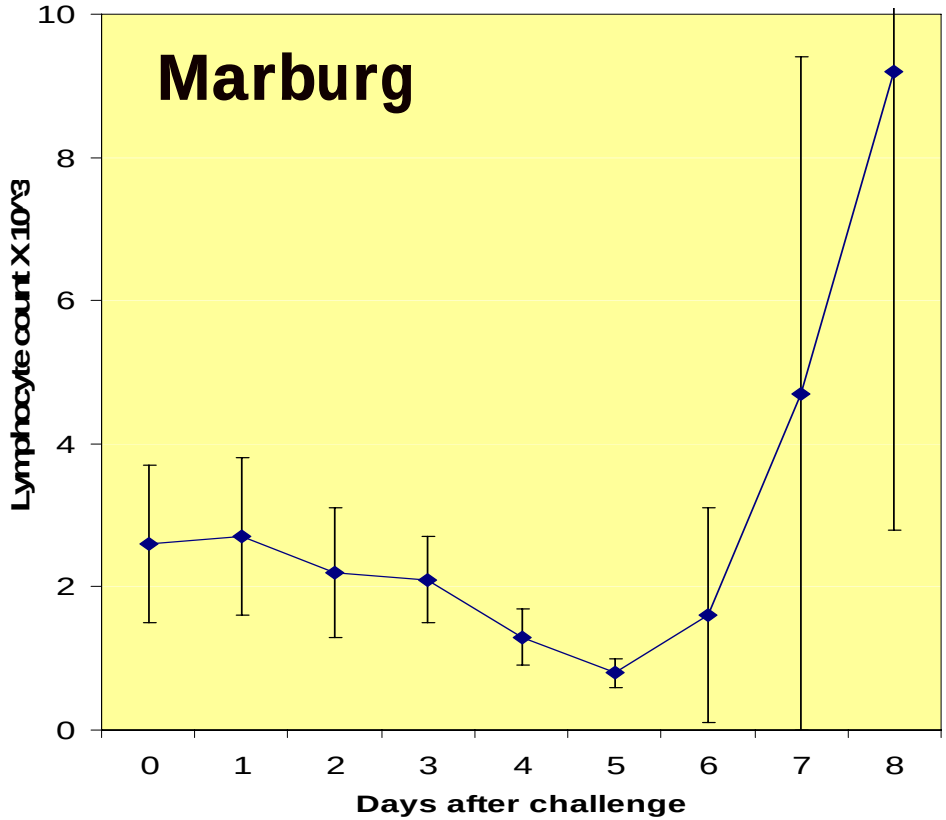






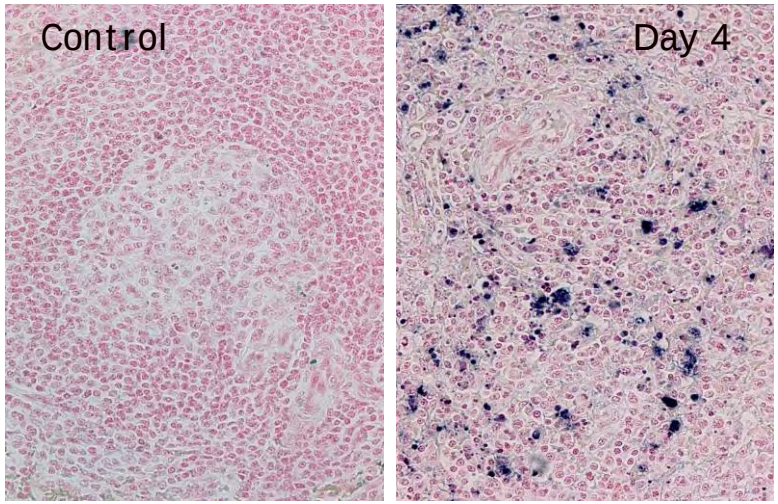
Lymphopenia



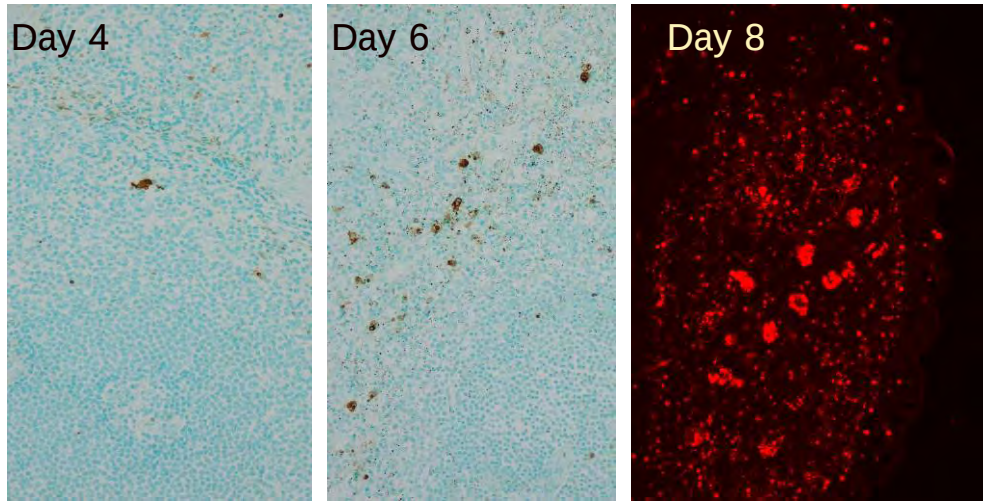


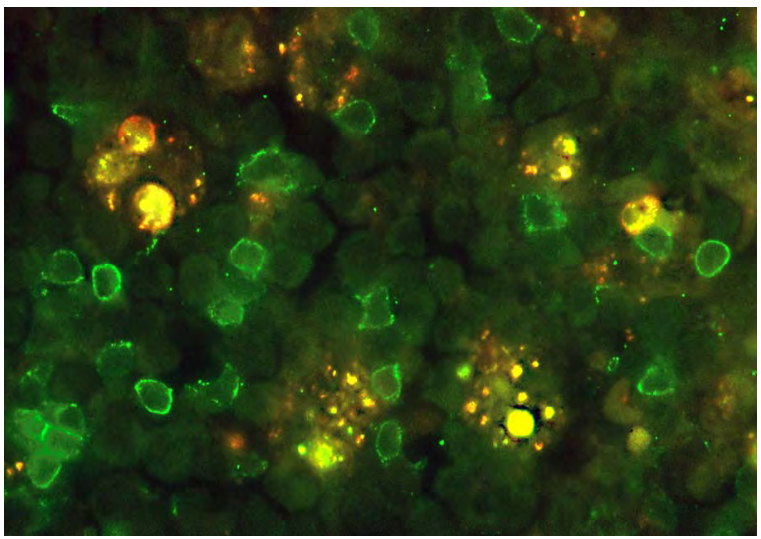
Lymphocyte Apoptosis -Filoviruses

Ebola

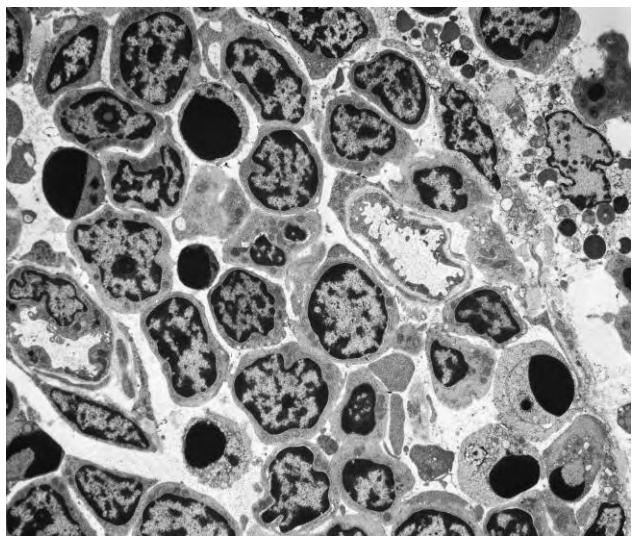


Marburg

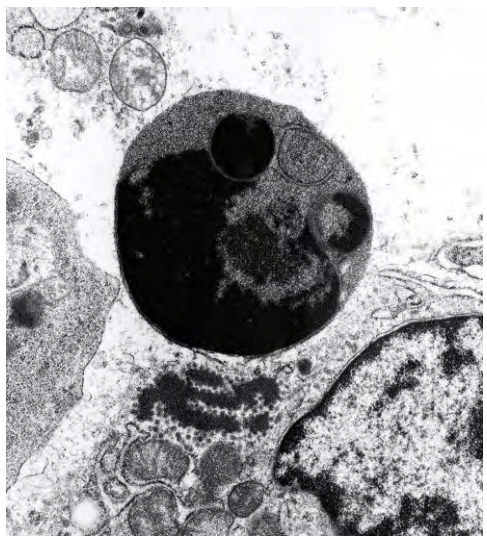




Ebola



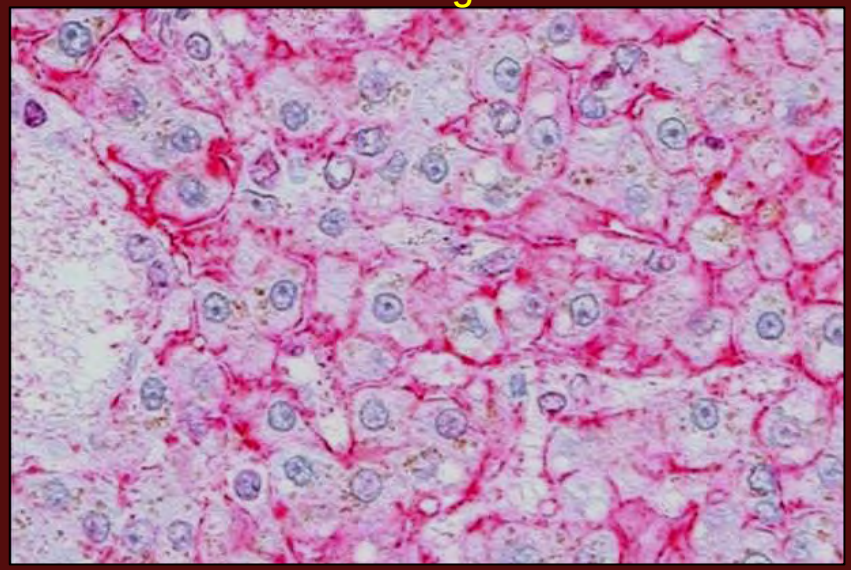
Ebola



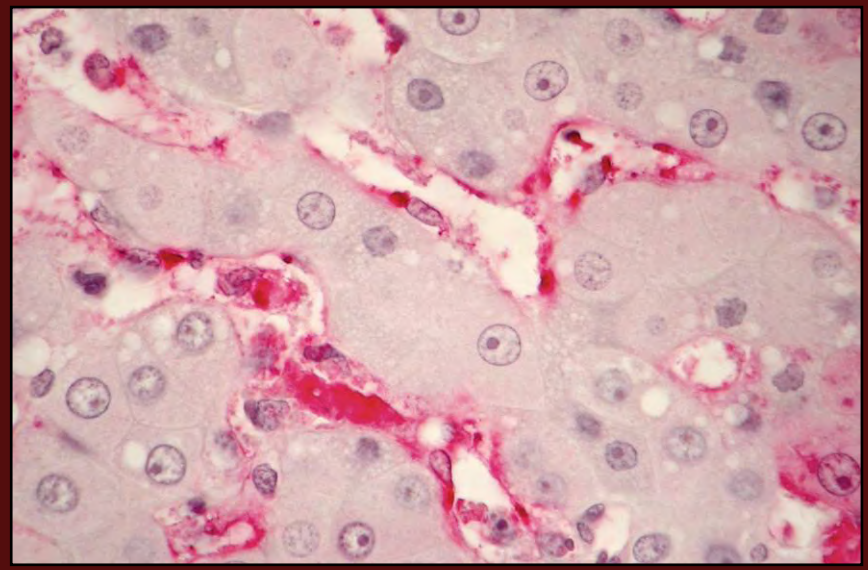
Marburg

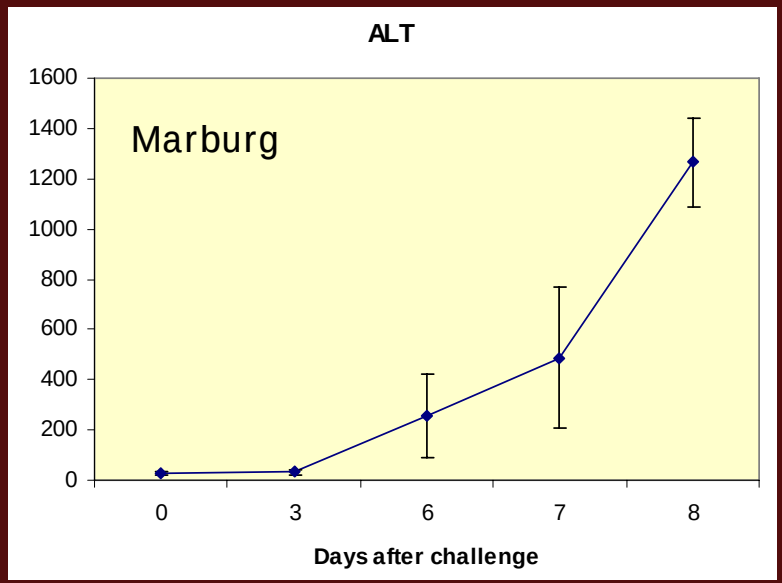
LIVER

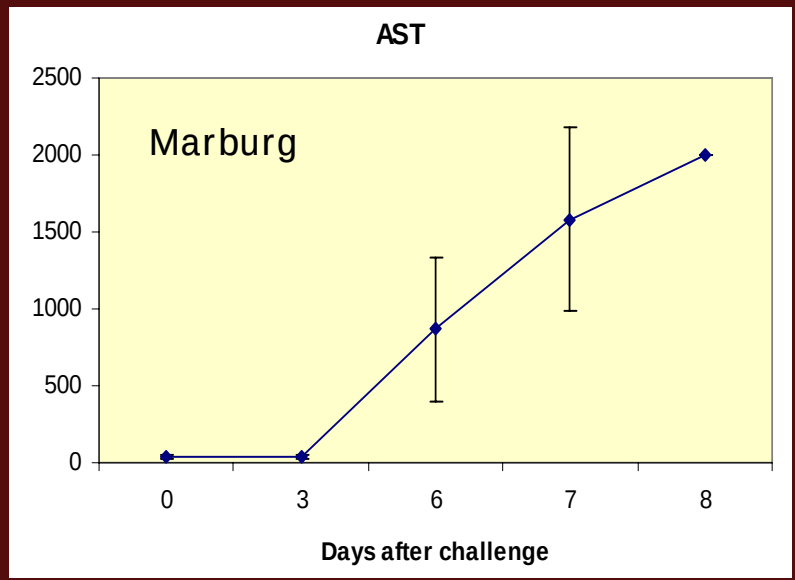
Marburg



Ebola



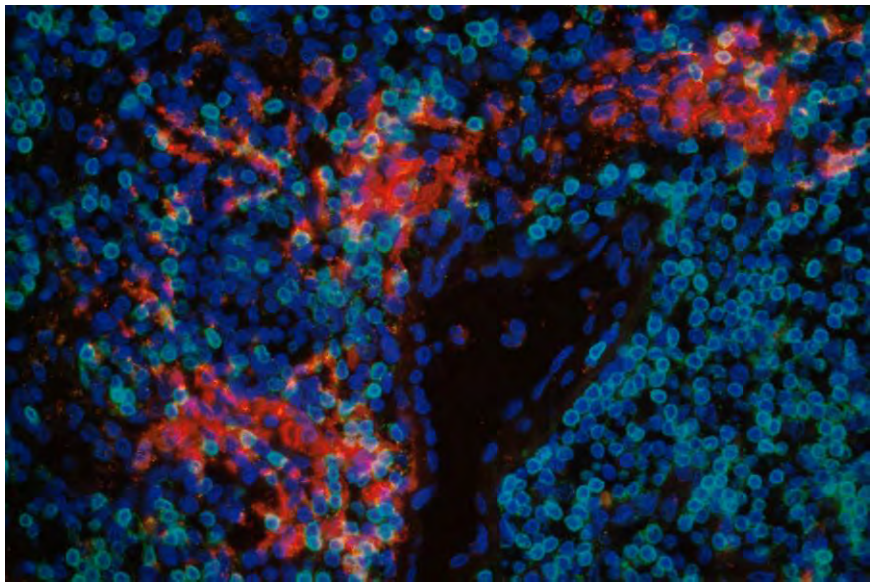


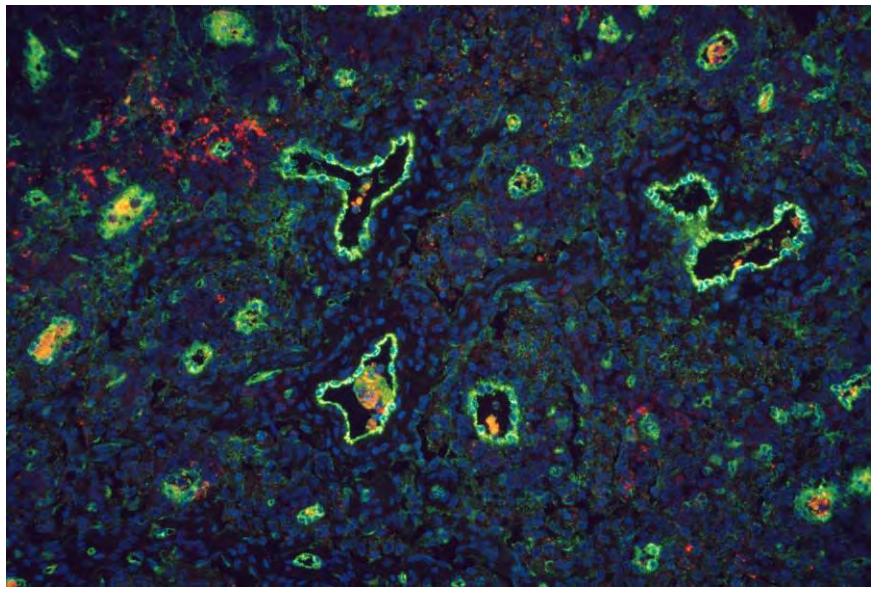


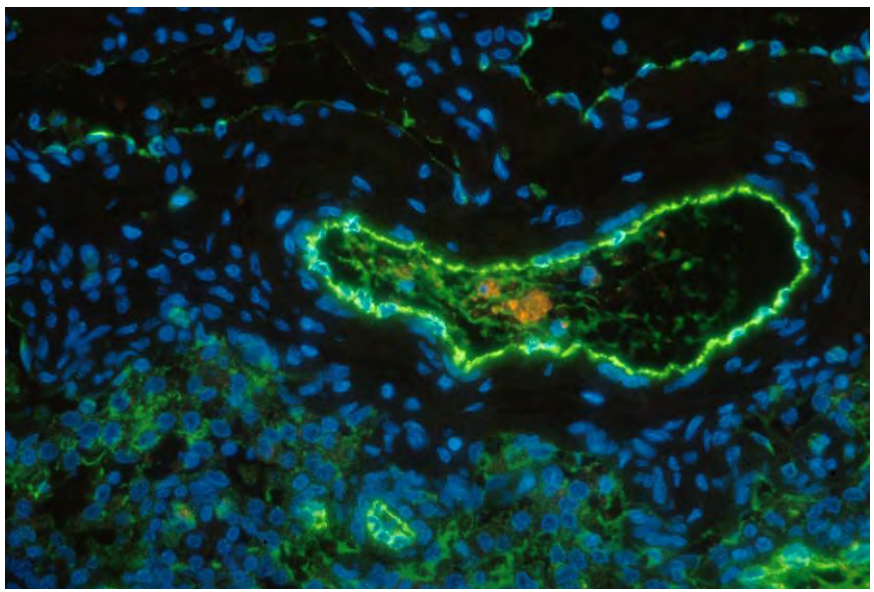
Disseminated Intravascular Coagulation (DIC)

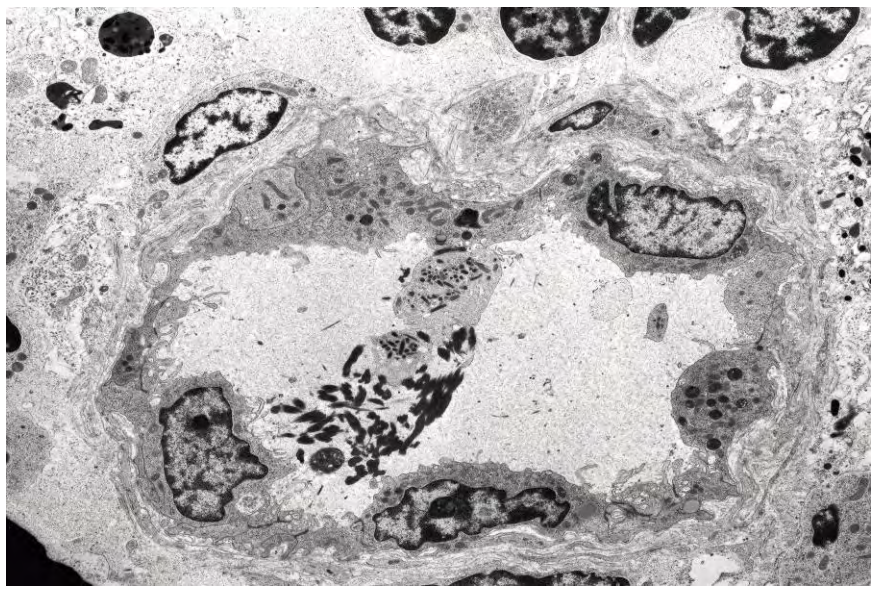
- Two major mechanisms trigger DIC:
  - widespread injury to endothelial cells
  - release of tissue factor or thromboplastic substances into the circulation
- Consequences of DIC:
  - Widespread deposition of fibrin within the microcirculation may lead to ischemia and/or hemolytic anemia resulting from fragmentation of RBCs as they squeeze through narrowed vasculature
  - Hemorrhagic diathesis resulting from consumption of platelets and clotting factors and activation of plasminogen

Lack of evidence for role of endothelial cells as a trigger for DIC

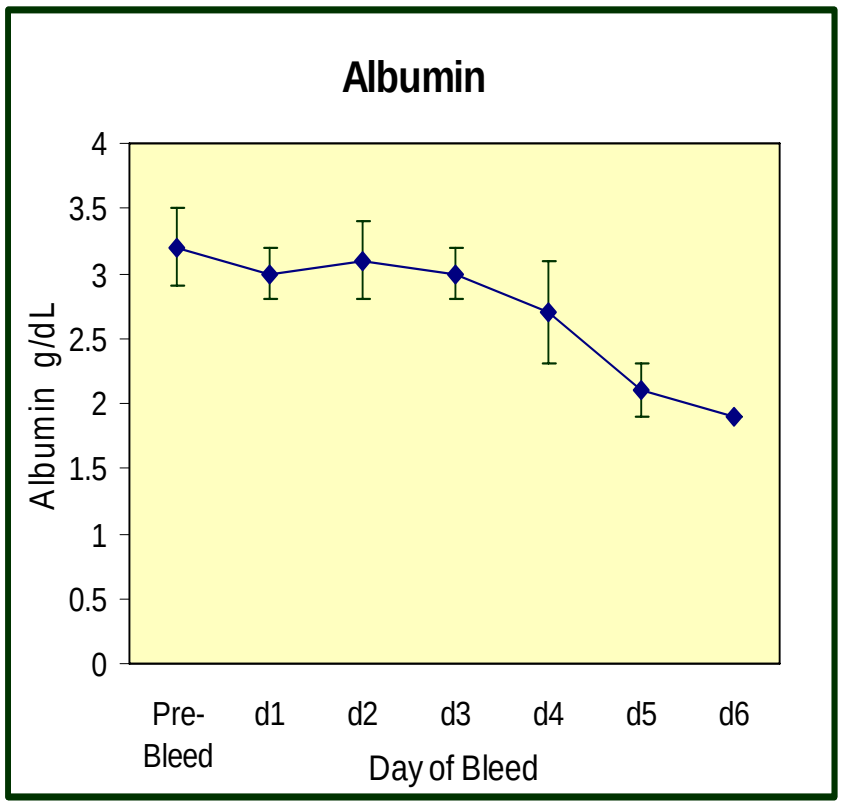


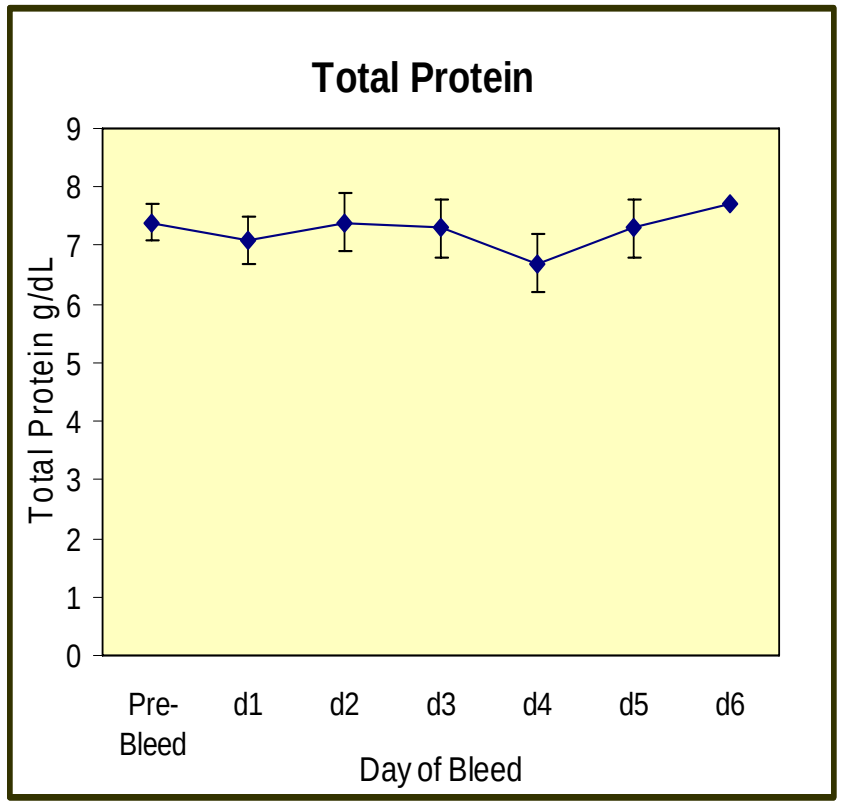




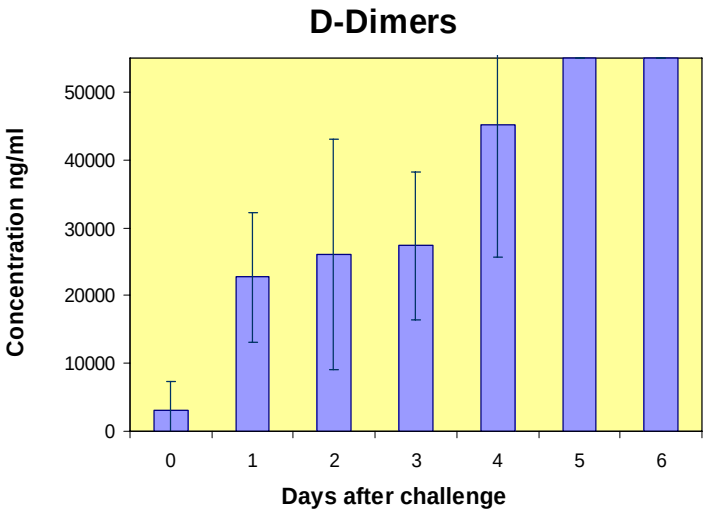


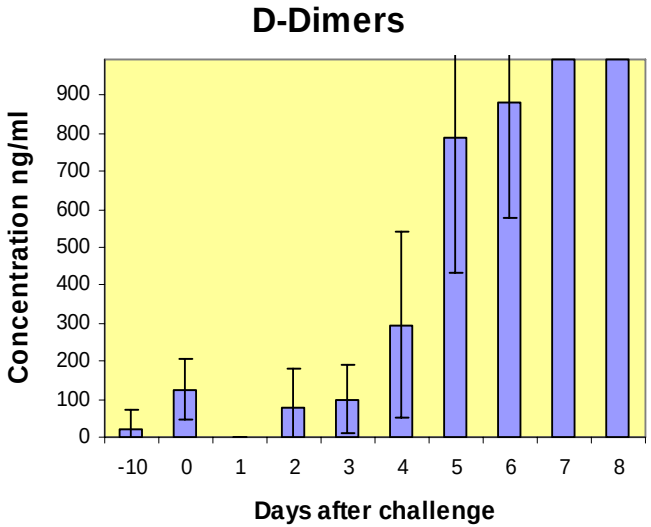
Serum Proteins in Ebola-Infected Monkeys

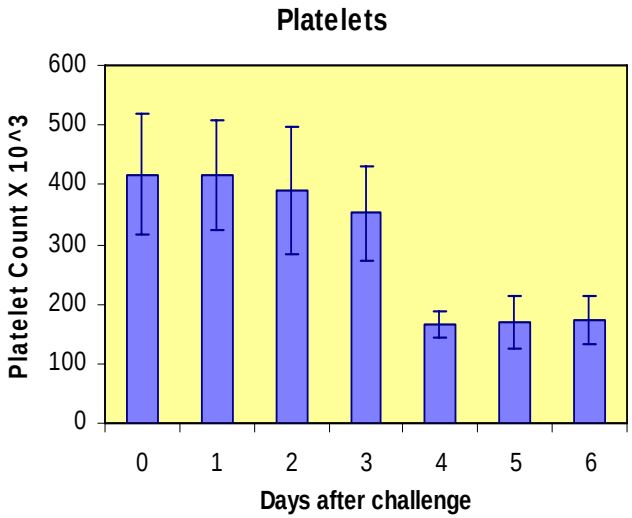


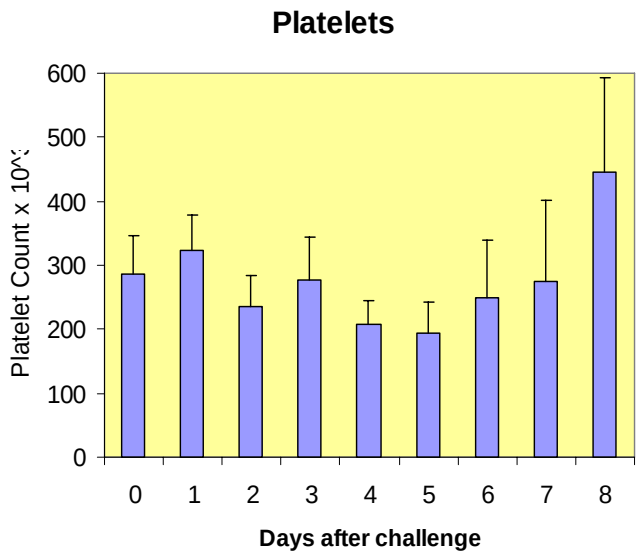


Coagulopathy EBOV vs MARV-Infected NHPs

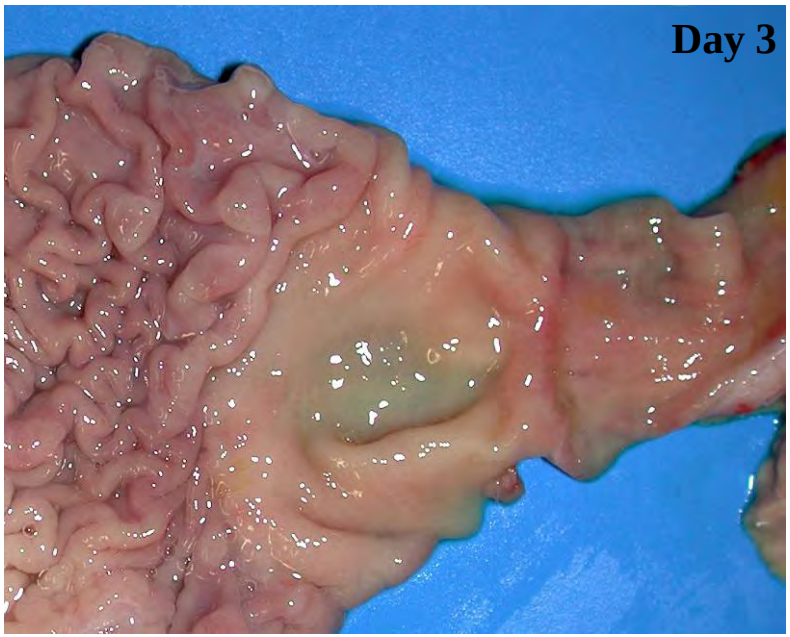


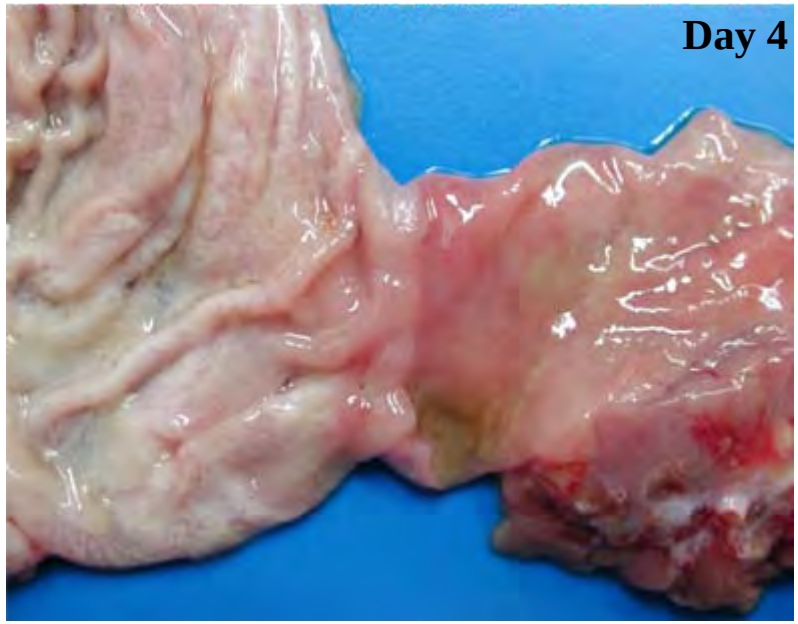


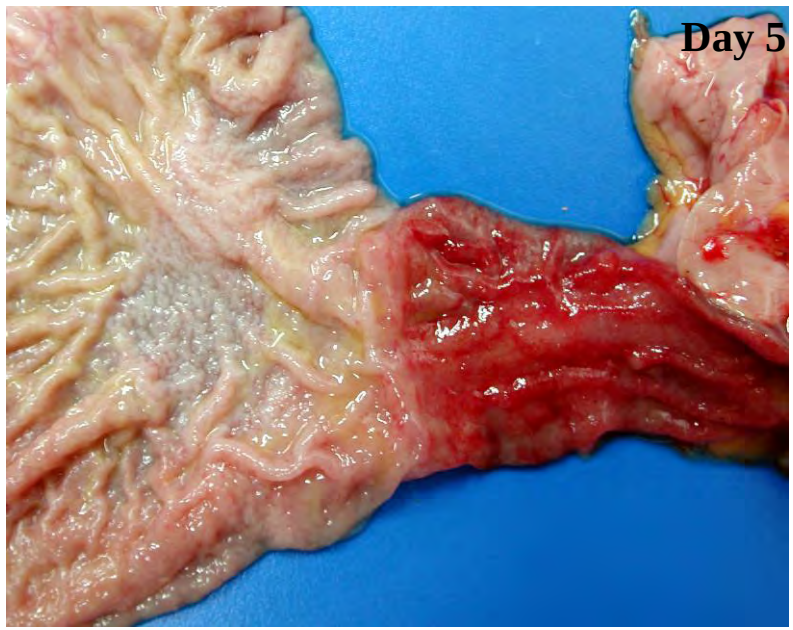




Duodenal Lesion

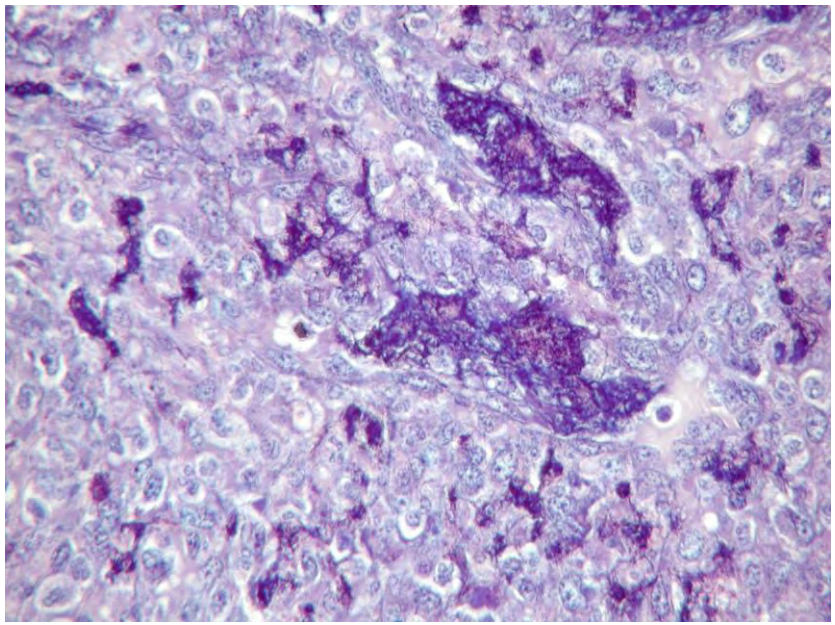




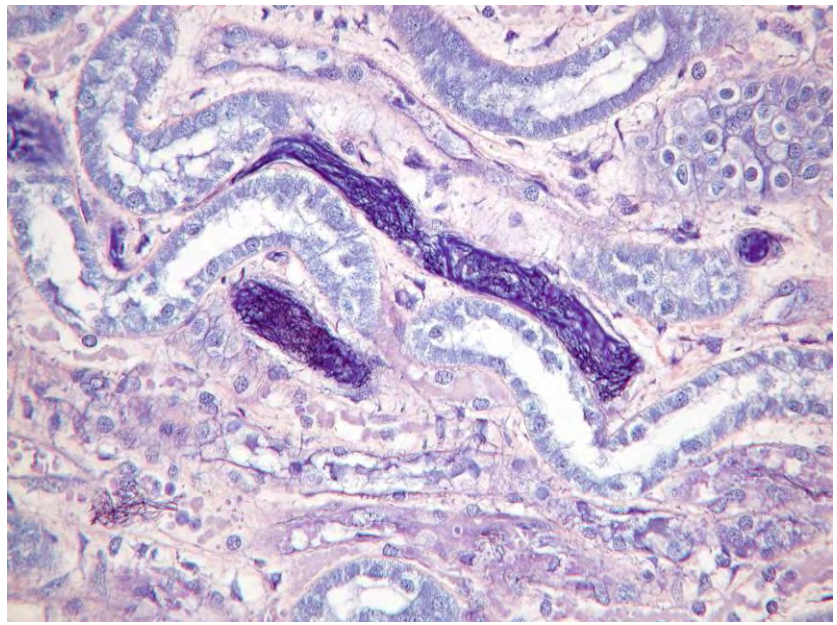


Gastroduodenal junction, d3-5 PE. Progressive hemorrhage/congestion of the proximal duodenum beginning at the pyloric sphincter and extending distally through the duodenum.

Fibrin deposition in EBOV-infected NHPs



Spleen – day 4



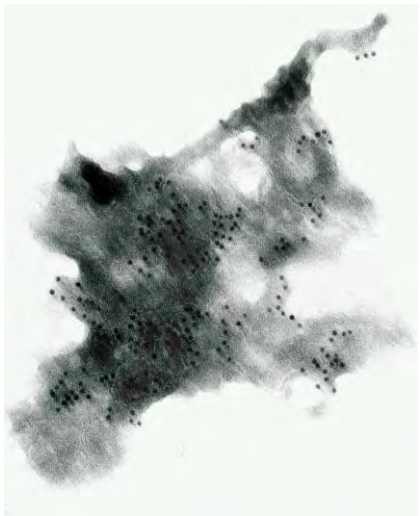
Kidney – day 4

Disseminated Intravascular Coagulation (DIC)

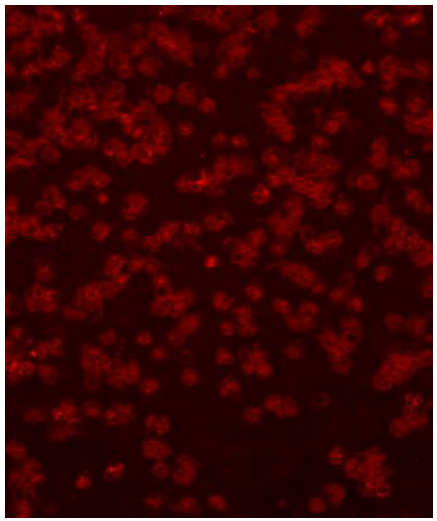
- Two major mechanisms trigger DIC:
  - widespread injury to endothelial cells
  - release of tissue factor or thromboplastic substances into the circulation
- Consequences of DIC:
  - widespread deposition of fibrin within the microcirculation may lead to ischemia and/or hemolytic anemia resulting from fragmentation of RBCs as they squeeze through narrowed vasculature
  - hemorrhagic diathesis resulting from consumption of platelets and clotting factors and activation of plasminogen

The Role of Tissue Factor in Ebola Infections

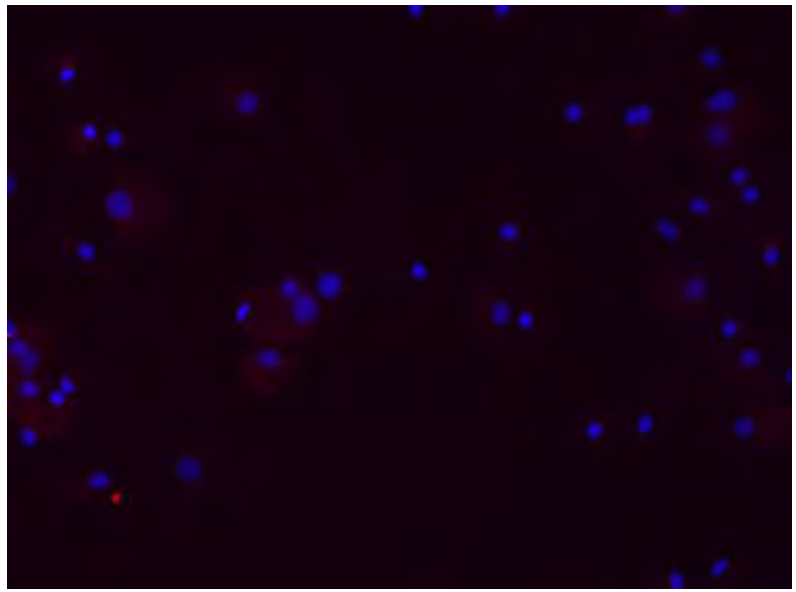
TF+ microparticles (NHP)



TF+ PBMC (NHP)



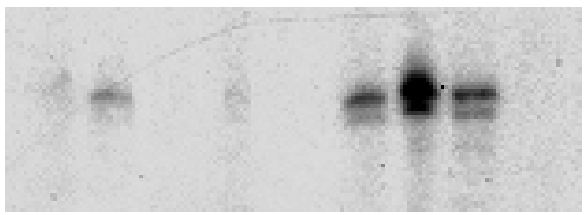
Primary human monocytes/macrophages



Primary human monocytes/macrophages

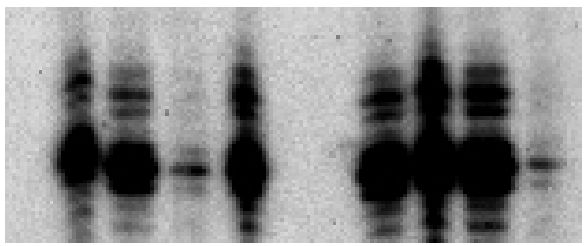
Control Ebola-infected

Tissue Factor

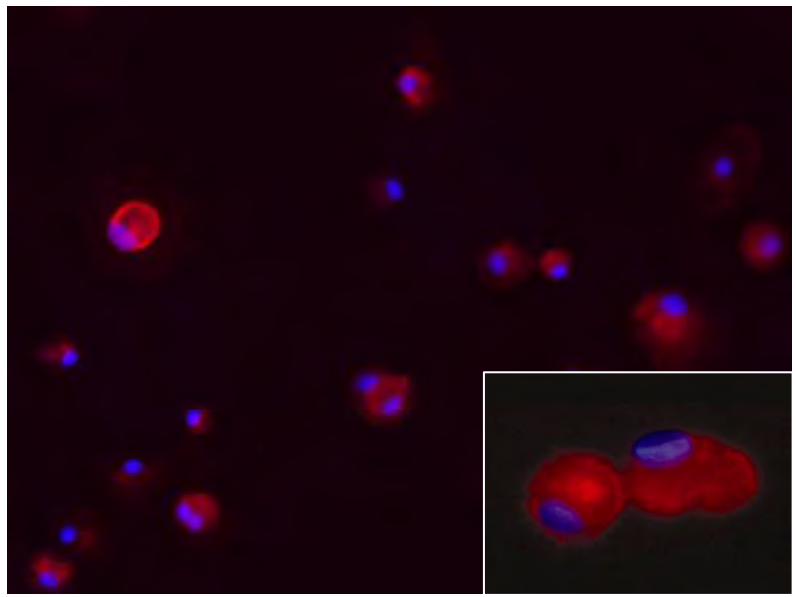


L32

GAPDH



1 24 96 48 1 24 48 96



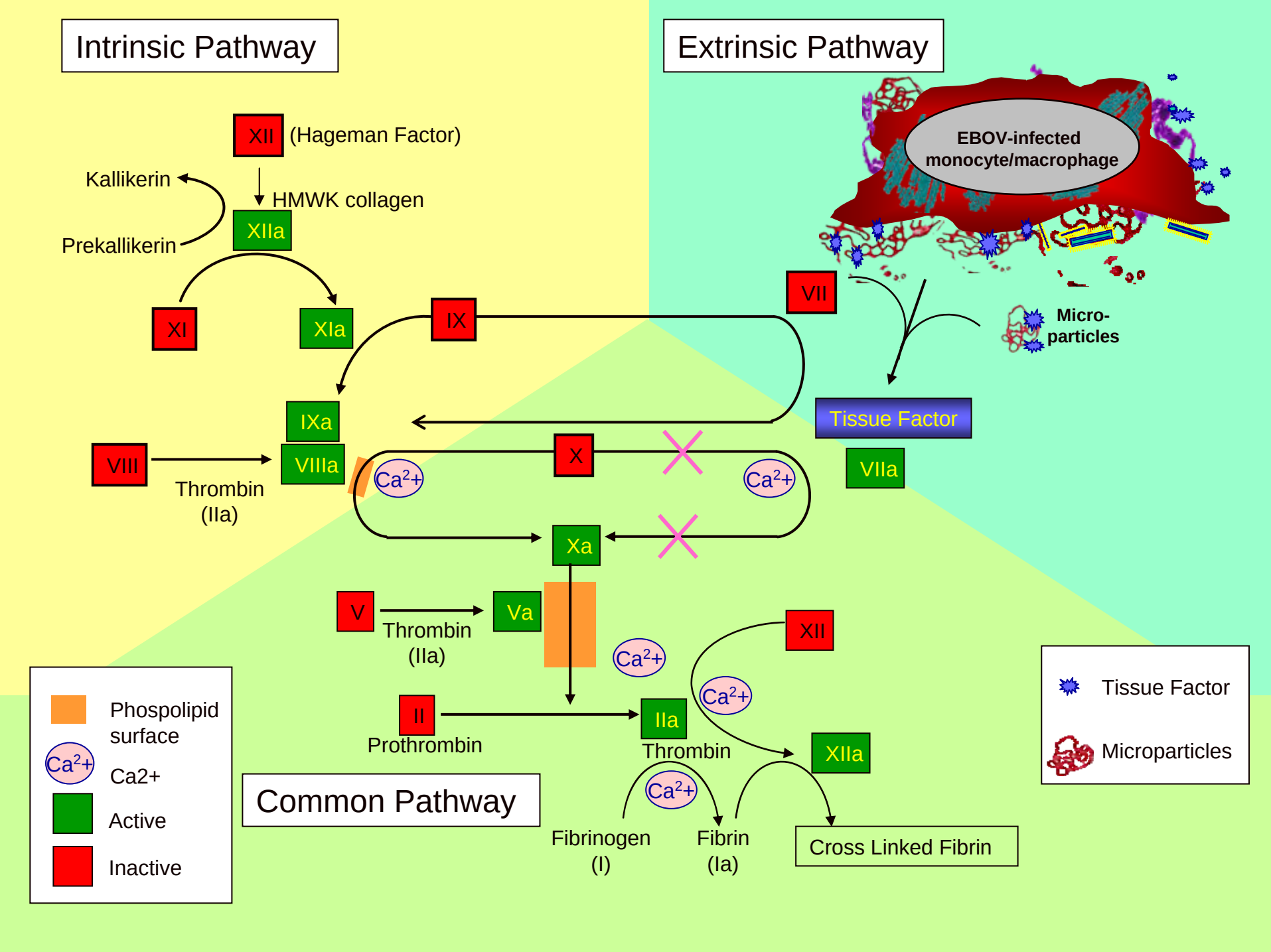
Marburg HF versus and Ebola HF in cynomolgus monkeys

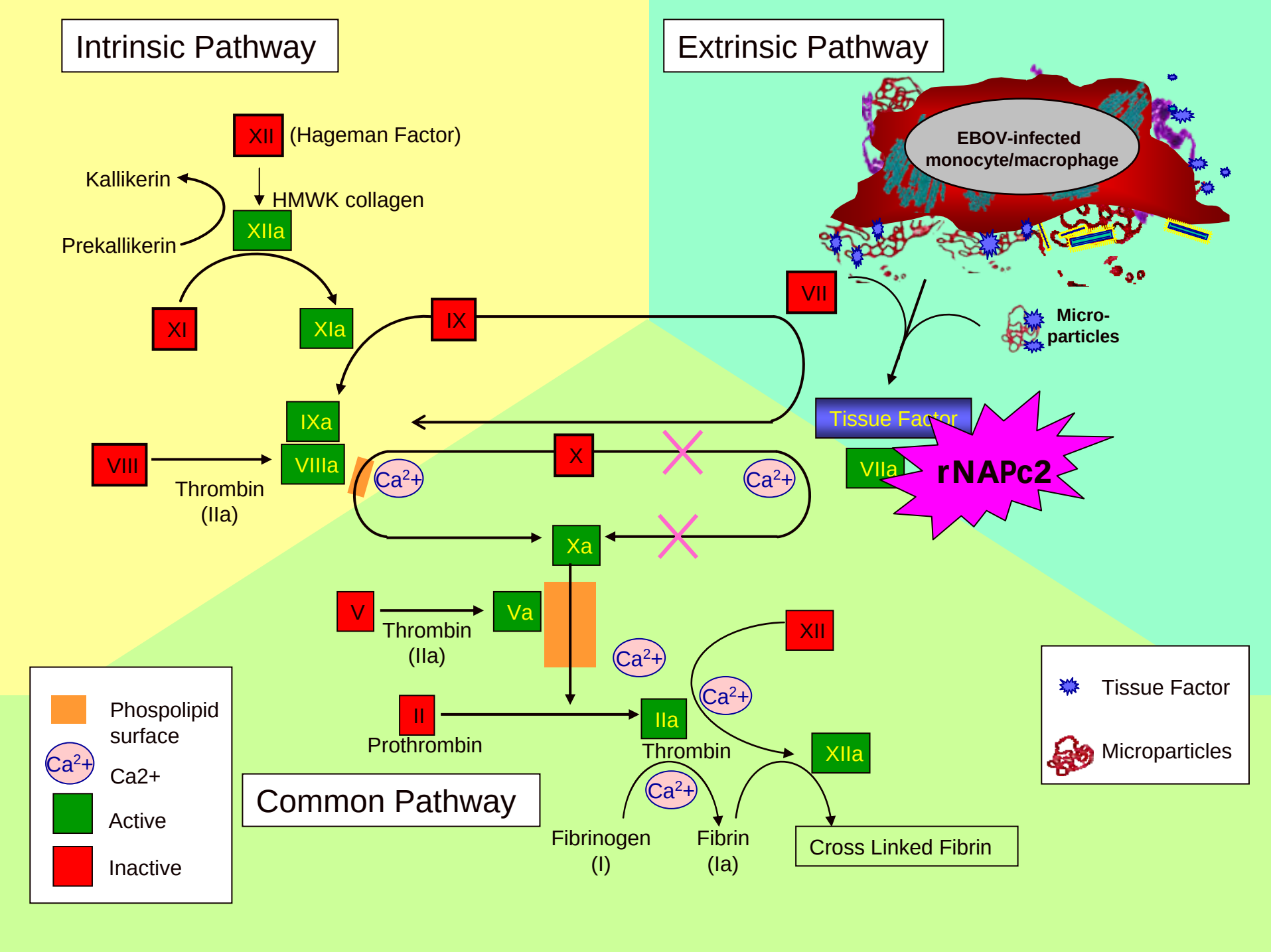
Similarities

- Early target cells: Monocytes/macrophages and dendritic cells
- Loss of lymphocytes in circulation and in lymphoid tissues by apoptosis
- Increased plasma levels of proinflammatory cytokines/chemokines, e.g., IL-6, MCP-1

Differences

- Fibrin deposits in tissues are less prominent in MARV HF
- Thrombocytopenia is not as dramatic in MARV HF than in EBOV HF
- Temporal difference in disease course with many changes in biomarkers in MARV HF occurring at later time points (closer to death)
- Liver more involved in MARV HF than Ebola


Intrinsic Pathway
Extrinsic Pathway
XII
(Hageman Factor)
Kallikerin
HMWK collagen
Prekallikerin
XIIa
XI
XIa
IX
IXa
VIII
VIIIa
Thrombin
(IIa)
Ca2+
X
Xa
V
Va
Thrombin
(IIa)
II
Prothrombin
IIa
Thrombin
Ca2+
XII
XIIa
Fibrinogen
(I)
Fibrin
(Ia)
Cross Linked Fibrin
Common Pathway
EBOV-infected
monocyte/macrophage
VII
Micro-
particles
Tissue Factor
VIIa
Phospolipid
surface
Ca2+
Active
Inactive
Tissue Factor
Microparticles


Intrinsic Pathway
Extrinsic Pathway
XII (Hageman Factor)
Kallikerin
HMWK collagen
Prekallikerin
XIIa
XI
XIa
IX
IXa
VIII
VIIIa
Thrombin
(IIa)
Ca2+
X
Xa
V
Va
Thrombin
(IIa)
II
Prothrombin
IIa
Thrombin
Ca2+
XII
XIIa
Fibrinogen
(I)
Fibrin
(Ia)
Cross Linked Fibrin
Common Pathway
EBOV-infected
monocyte/macrophage
VII
Micro-
particles
Tissue Factor
VIIa
rNAPc2
Phospolipid
surface
Ca2+
Active
Inactive
Tissue Factor
Microparticles

Articles

Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys

Thomas W Geisbert, Lisa E Hensley, Peter B Jahrling, Tom Larsen, Joan B Geisbert, Jason Paragas, Howard A Young, Terry M Fredeking, William E Rote, George P Vlasuk

Summary

Background Infection with the Ebola virus induces overexpression of the procoagulant tissue factor in primate monocytes and macrophages, suggesting that inhibition of the tissue-factor pathway could ameliorate the effects of Ebola haemorrhagic fever. Here, we tested the notion that blockade of fVIIa/tissue factor is beneficial after infection with Ebola virus.

Methods We used a rhesus macaque model of Ebola haemorrhagic fever, which produces near 100% mortality. We administered recombinant nematode anticoagulant protein c2 (rNAPc2), a potent inhibitor of tissue factor-initiated blood coagulation, to the macaques either 10 min (n=6) or 24 h (n=3) after a high-dose lethal injection of Ebola virus. Three animals served as untreated Ebola virus-positive controls. Historical controls were also used in some analyses.

Findings Both treatment regimens prolonged survival time, with a 33% survival rate in each treatment group. Survivors are still alive and healthy after 9 months. All but one of the 17 controls died. The mean survival for the six rNAPc2-treated macaques that died was 11·7 days compared with 8·3 days for untreated controls (p=0·0184). rNAPc2 attenuated the coagulation response as evidenced by modulation of various important coagulation factors, including plasma D dimers, which were reduced in nearly all treated animals; less prominent fibrin deposits and intravascular thromboemboli were observed in tissues of some animals that succumbed to Ebola virus. Furthermore, rNAPc2 attenuated the proinflammatory response with lower plasma concentrations of interleukin 6 and monocyte chemoattractant protein-1 (MCP-1) noted in the treated than in the untreated macaques.

Interpretation Post-exposure protection with rNAPc2 against Ebola virus in primates provides a new foundation for therapeutic regimens that target the disease process rather than viral replication.

Lancet 2003; **362:** 1953–58
See Commentary

Virology Division (T W Geisbert PhD, L E Hensley PhD, J B Geisbert, J Paragas PhD), **Headquarters** (P B Jahrling PhD), **and Pathology Division** (T Larsen DVM), **US Army Medical Research Institute of Infectious Diseases (USAMRIID), Fort Detrick, MD, USA; Cellular and Molecular Immunology Section, Laboratory of Experimental Immunology, NCI-FCRDC, Frederick, MD, USA** (H A Young PhD); **Antibody Systems, Hurst, TX, USA** (T M Fredeking PhD); **and Corvas International, San Diego, CA, USA** (W E Rote PhD, G P Vlasuk PhD)

Correspondence to: Dr Thomas W Geisbert, Virology Division, USAMRIID, Fort Detrick, MD 21702, USA
(e-mail: tom.geisbert@amedd.army.mil)

Introduction

Ebola virus causes severe haemorrhagic fever in primates.[1,2] Acute mortality caused by the Zaire species of Ebola virus has been about 80% in outbreaks in human beings[1] and nearly 100% in monkey models of the genus macaca.[2] There are no effective treatments for Ebola virus haemorrhagic fever. Various therapeutic strategies protect rodents from lethal Ebola haemorrhagic fever; however, these strategies have not proven effective in non-human primates,[3–6] suggesting important pathogenic differences between these models.[2,7]

The disease triggered in primates is thought to involve inappropriate or maladaptive host responses, and includes development of coagulation abnormalities not evident in rodents. Although the coagulopathy seen in Ebola haemorrhagic fever is probably caused by multiple factors, data suggest tissue factor plays an important part in triggering the coagulation abnormalities that characterise infections in primates.[8] The exposure of cells that express tissue factor on their surfaces to flowing blood is sufficient to initiate coagulation.[9] Expression of tissue factor can be induced in the endothelium and in monocytes in vitro by various agonists, even though these cells do not constitutively express tissue factor.[9] Previously, we have shown[8] that Ebola virus induces overexpression of tissue factor in primate monocytes and macrophages, and that overexpression depends on viral replication. Overexpression of tissue factor is one of the leading causes of disseminated intravascular coagulation and thrombosis-related organ failure.[10] Therefore, we reasoned that by blocking the pathway leading from the formation of fVIIa/tissue factor to thrombin, we might alter the disease pathogenesis in Ebola virus infections of non-human primates, with the hope that this approach might be useful in augmenting strategies that have protected rodents from lethal infection.

Recombinant nematode anticoagulant protein c2 (rNAPc2) is an 85-aminoacid protein that directly inhibits the fVIIa/tissue factor complex by a unique mechanism that requires initial binding of rNAPc2 to activated or zymogen factor X.[11] The antithrombotic potential of fVIIa/tissue factor inhibition by rNAPc2 has been shown in phase II trials in orthopaedic surgery[12] and coronary revascularisation.[13] We, therefore, used an established rhesus macaque model of Ebola haemorrhagic fever[2] to test the notion that blockade of fVIIa/tissue factor is beneficial after infection with Ebola virus.

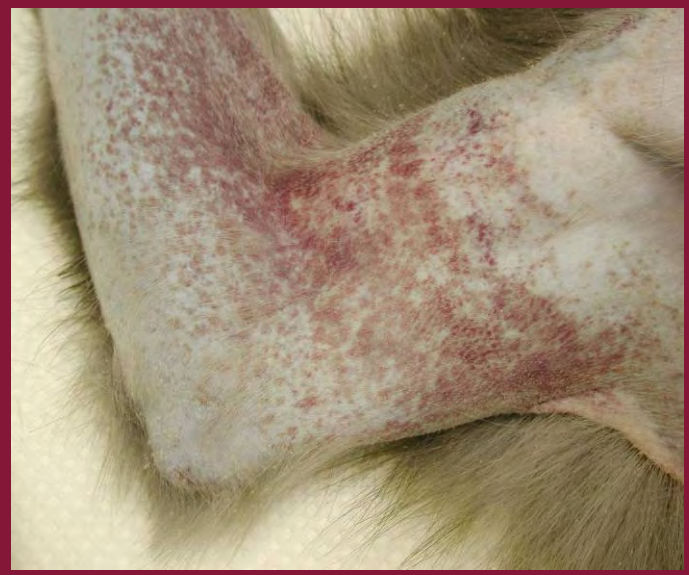
Methods

Animals

We inoculated healthy adult rhesus macaques (*Macaca mulatta*) by intramuscular injection with 0·5 mL of viral stock that contained 1000 plaque forming units (PFU) of Ebola virus (Zaire 95 isolate).[3]

Our research was undertaken in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving

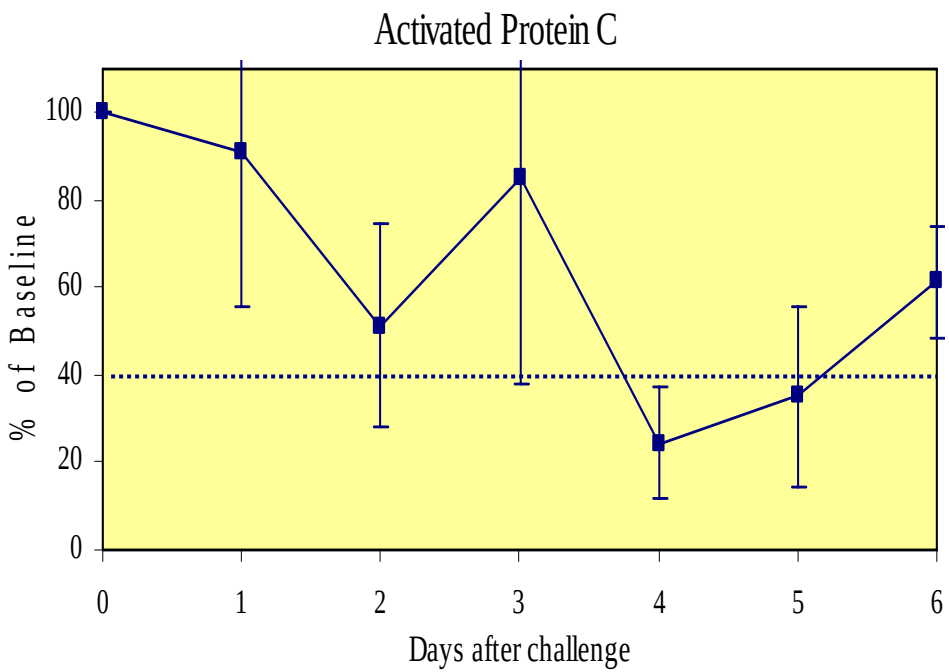
rNAPc2-treated d10



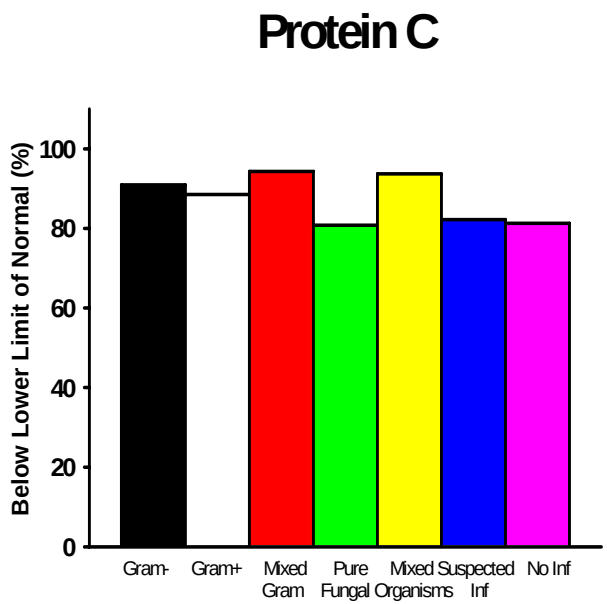
Placebo control – d9

Plasma Levels of Protein C

EBOV-Infected Macaques



Human Severe Sepsis Patients



- A rapid decline in plasma protein C levels occurs in EBOV infections
- This decline is concomitant with disease progression
- Decreases are observed before the development of clinical disease
- A drop of 40% of APC in severe sepsis is a significant predictor of poor outcome

Recombinant human activated protein C (rhAPC) (Drotrecogin alfa [activated]; Xigris®)

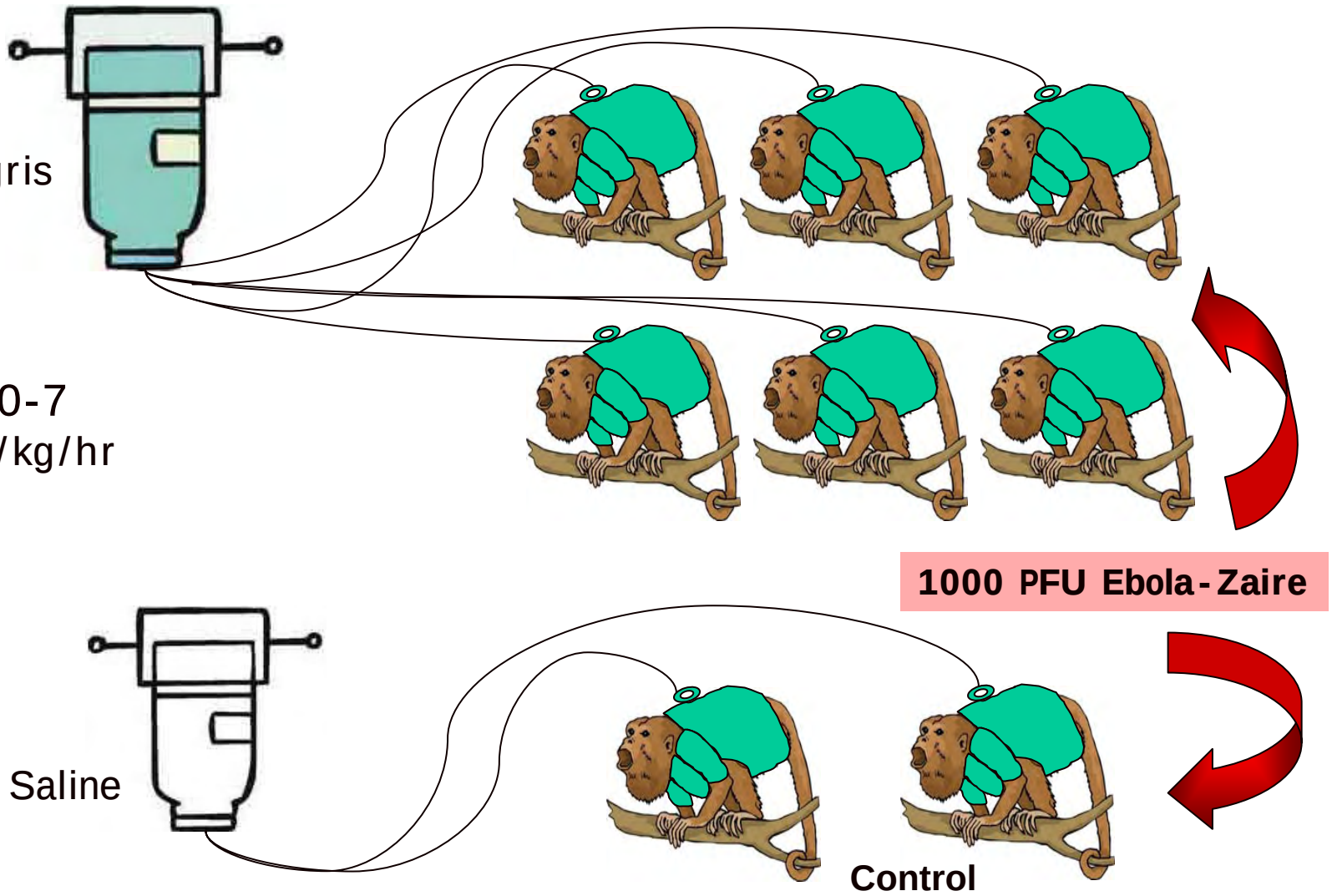
- rhAPC is a complex 60 Kd serine protease with 4 types of post-translational modifications
- The approved Xigris dose for severe sepsis is 24 μg/kg/hr for 96 hrs
  - Highest NOAEL* from toxicology (in monkeys) and in phase 1 studies is 48 μg/kg/hr
- The antithrombotic activity of APC is highly species specific (i.e., rhAPC has much lower antithrombotic activity in nonhuman primates)
  - NHP are the most relevant species for testing Xigris besides human

Could rhAPC have utility in treating Ebola HF?
Activated Protein C has a very short half life ~ 13 min

- This will require the continuous administration of drug to NHP under a BSL-4 setting

* NOAEL – No Observed Adverse Event Level

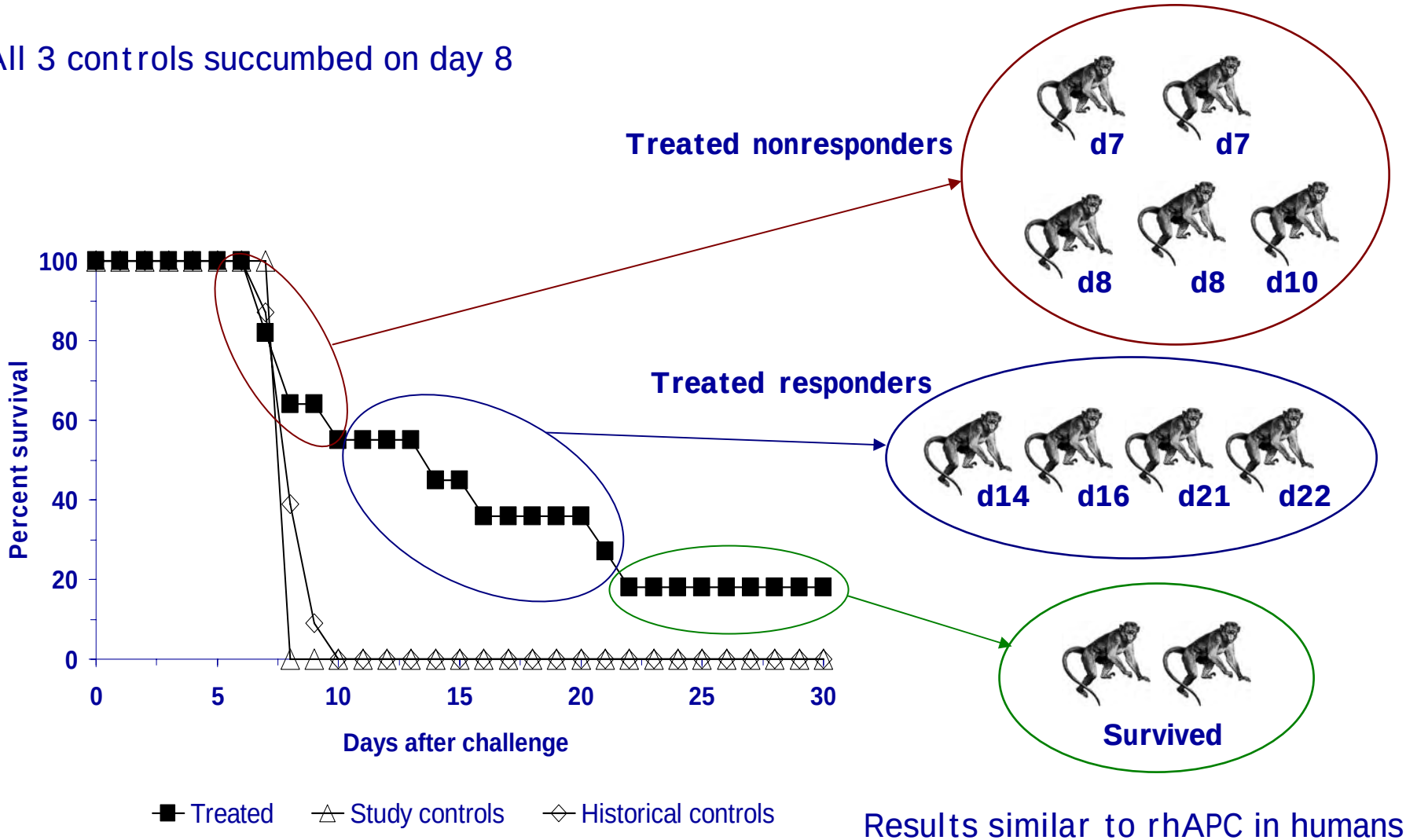
Recombinant Human Activated Protein C Treatment Study



- 3 separate studies performed
- Controls n=3; Treated n=11

Survival

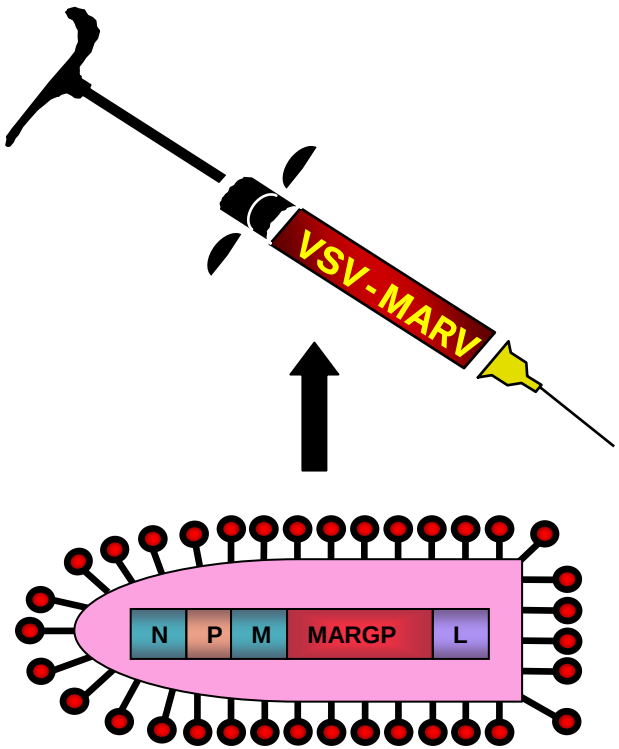
All 3 controls succumbed on day 8



Mean survival = 12.6 days versus 8.3 days for historical controls (P= 0.049)

Results similar to rhAPC in humans (relative reduction in risk of death is 19.4% and absolute reduction in risk of death is 6.1%)

A Vaccine as a Postexposure Treatment?



Postexposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment

Kathleen M Daddario-DiCaprio, Thomas W Geisbert, Ute Ströher, Joan B Geisbert, Allen Grolla, Elizabeth A Fritz, Lisa Fernando, Elliott Kagan, Peter B Jahrling, Lisa E Hensley, Steven M Jones, Heinz Feldmann

Summary

Background Effective countermeasures are urgently needed to prevent and treat infections caused by highly pathogenic and biological threat agents such as Marburg virus (MARV). We aimed to test the efficacy of a replication-competent vaccine based on attenuated recombinant vesicular stomatitis virus (rVSV), as a postexposure treatment for MARV haemorrhagic fever.

Methods We used a rhesus macaque model of MARV haemorrhagic fever that produced 100% lethality. We administered rVSV vectors expressing the MARV Musoke strain glycoprotein to five macaques 20–30 min after a high-dose lethal injection of homologous MARV. Three animals were MARV-positive controls and received non-specific rVSV vectors. We tested for viraemia, undertook analyses for haematology and serum biochemistry, and measured humoral and cellular immune responses.

Findings All five rhesus monkeys that were treated with the rVSV MARV vectors as a postexposure treatment survived a high-dose lethal challenge of MARV for at least 80 days. None of these five animals developed clinical symptoms consistent with MARV haemorrhagic fever. All the control animals developed fulminant disease and succumbed to the MARV challenge by day 12. MARV disease in the controls was indicated by: high titres of MARV (10^3–10^5 plaque-forming units per mL); development of leucocytosis with concurrent neutrophilia at end-stage disease; and possible damage to the liver, kidney, and pancreas.

Interpretation Postexposure protection against MARV in non-human primates provides a paradigm for the treatment of MARV haemorrhagic fever. Indeed, these data suggest that rVSV-based filoviral vaccines might not only have potential as preventive vaccines, but also could be equally useful for postexposure treatment of filoviral infections.

Published Online
April 27, 2006
DOI:10.1016/S0140-6736(06)68546-2

See also Online/Comment
DOI:10.1016/S0140-6736(06)68547-4

Virology Division, US Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD, USA (K M Daddario-DiCaprio, T W Geisbert PhD, J B Geisbert, E A Fritz PhD, L E Hensley PhD); **Uniformed Services University of the Health Sciences, Bethesda, MD, USA** (K M Daddario-DiCaprio, T W Geisbert, Prof E Kagan MD); **Special Pathogens Program, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada** (S M Jones PhD, U Ströher PhD, A Grolla, L Fernando, H Feldmann MD); **Department of Medical Microbiology** (S M Jones, U Ströher, H Feldmann) **and Department of Immunology** (S M Jones), **University of Manitoba, Winnipeg, MB, Canada; and Integrated Research Facility, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA** (P B Jahrling)

Correspondence to:
Dr Thomas W Geisbert
tom.geisbert@amedd.army.mil

Introduction

Marburg virus (MARV) is a filovirus that causes a severe, and often fatal, haemorrhagic disease, for which there is currently no vaccine or therapy approved for human use. The reported potential of MARV as a biological weapon[1] and the recent attention drawn to outbreaks of emerging and re-emerging viruses, such as the 2004–05 epidemic of MARV haemorrhagic fever in Angola,[2] have greatly increased public recognition of this deadly pathogen.

The recent MARV outbreak in Angola, with case fatality rates approaching 90%, calls attention to the urgent need for effective countermeasures against filoviruses. So far, the only available form of treatment for MARV haemorrhagic fever is intensive supportive care. The development of effective treatments and therapies for the disease has been a continuing challenge since the disease was first recorded in 1967.[3] The requirement for biosafety level (BSL) 4 containment has been a major impediment towards the development of MARV therapeutics.

Guineapig and non-human primate models have been developed for MARV haemorrhagic fever.[4–11] Although these models have been used in several studies to investigate candidate vaccines, few studies have examined postexposure interventions. Several immunomodulatory drugs, including desferal, ridostin, and polyribonate, were investigated in guineapig models of experimental MARV infection; some protection and slight increases in mean time to death were recorded.[5,11] Despite the ability of several of these drugs to induce protective responses in guineapigs, the efficacy and action of these immunomodulators in non-human primates has yet to be determined. Furthermore, interferon has shown no substantial therapeutic potential against MARV infection in non-human primate models; similarly, ribavirin has shown no effect in guineapig models.[5,9,11]

Despite the slow progress in treatment development for MARV haemorrhagic fever, important advances have been made in the development of preventive vaccines against MARV and another filovirus, Ebola virus (EBOV). In particular, several recombinant vaccines have shown promising findings in non-human primate models of filoviral haemorrhagic fever, including vaccines based on recombinant adenoviruses[12,13] and recombinant alphaviruses.[8] We previously described the generation and assessment of a live, attenuated, recombinant vesicular stomatitis virus (rVSV) expressing the transmembrane glycoprotein of MARV (VSVΔG/MARVGP)[10,14] and showed that vaccination with this vector completely protected non-human primates against a lethal MARV challenge.[10] The rVSV vaccine platform

Common Denominators of Survival in Filovirus-Infected Macaques

- Maintenance of D-dimer levels
- Maintenance of protein C activity (> 50%)
- Maintenance of levels of proinflammatory / procoagulant cytokines (e.g., IL-6)
- Low viral load

nature medicine

Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses

Steven M Jones[1,2,9], Heinz Feldmann[1,3,9], Ute Ströher[1,3], Joan B Geisbert[4], Lisa Fernando[1], Allen Grolla[1], Hans-Dieter Klenk[5], Nancy J Sullivan[6], Viktor E Volchkov[7], Elizabeth A Fritz[4], Kathleen M Daddario[8], Lisa E Hensley[4], Peter B Jahrling[4] & Thomas W Geisbert[4]

Vaccines and therapies are urgently needed to address public
and bio
virus (E
replica
on atte
express
A singl
elicited
primat
vaccine
and no
of illne
induce
all vacc
stronge
of EBO
protect
vaccine
animal

Ebola v
Filoviri
hemorr
primate
in rece
cases in
in Ango
agents
vaccine
ated liv
expressi
VSV ∴

evaluated the utility of these rVSV vectors as candidate vaccines for

Photos courtesy of Fort Detrick

Electron micrographs of Ebola virus

BREAKTHROUGH!

Scientists have developed vaccines effective in monkeys against both the Marburg and Ebola viruses

By JAMES RADA | *News-Post Staff* | jrada@fredericknewspost.com

Staff photo by Sam Yu

Joan Geisbert, a lab technician at USAMRIID at Fort Detrick, works with Ebola virus in one of USAMRIID's bio safety level 4 labs in this photo from 2002. These labs are reserved for the most dangerous organisms. Scientists from USAMRIID and the Public Health Agency of Canada have recently developed vaccines against the Marburg and Ebola viruses.

FREDERICK — Last fall, after decades of civil war, Angolans faced a new enemy. This one was unseen and killed from within.

The new enemy is the Marburg virus. It can kill 90 percent of its infected victims quickly and painfully. It can cause internal organs to liquefy, skin to bubble up, and victims to weep tears of blood, according to Richard Preston in his book "The Hot Zone."

More than 215 people have died in Angola since the Marburg virus outbreak began last fall. While very little can be done to stop this outbreak, future "hot zones" may see some cooling with help from Frederick and Winnipeg, Canada.

Scientists from the U.S. Army Medical Research Institute of Infectious Diseases and the Public Health Agency of Canada have developed the first vaccines against both the Marburg and Ebola viruses that protect monkeys.

"We've made more progress in three years than we probably made in the previous 20," said Dr. Thomas Geisbert with the U.S. Army Medical Research Institute of Infectious Diseases at Fort Detrick.

Mr. Geisbert, along with Canadian researchers Dr. Heinz Feldmann and Dr. Steven Jones of PHAC's National Microbiology Laboratory, developed the vaccines after four years of work on the problem. This month's *Nature Medicine* journal published their study.

Ebola and Marburg viruses are emerging pathogens that cause hemorrhagic fever with high mortality rates in humans and non-human primates. Since monkeys develop hemorrhagic fever symptoms similar to humans, a safe and effective vaccine for monkeys shows promise for human use.

"When you see the tragedies these viruses cause, it's very frustrating that we can't do more to help people," said Mr. Feldmann, who has been providing on-site rapid diagnostic support to the cur-

(See BREAKTHROUGH A-11)

Development of a preventive vaccine for Ebola virus infection in primates

Nancy J. Sullivan*, Anthony Sanchez†, Pierre E. Rollin†, Zhi-yong Yang* & Gary J. Nabel*

** Vaccine Research Center, National Institutes of Health, 40 Convent Drive, MSC-3005, Bethesda, Maryland 20892, USA*
† Special Pathogens Branch, Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop G14, Atlanta, Georgia 30333, USA

Outbreaks of haemorrhagic fever caused by the Ebola virus are associated with high mortality rates that are a distinguishing feature of this human pathogen. The highest lethality is associated with the Zaire subtype, one of four strains identified to date[1,2]. Its rapid progression allows little opportunity to develop natural immunity, and there is currently no effective anti-viral therapy. Therefore, vaccination offers a promising intervention to prevent infection and limit spread. Here we describe a highly effective vaccine strategy for Ebola virus infection in non-human primates. A combination of DNA immunization and boosting with adenoviral vectors that encode viral proteins generated cellular and humoral immunity in cynomolgus macaques. Challenge with a lethal dose of the highly pathogenic, wild-type, 1976 Mayinga strain of Ebola Zaire virus resulted in uniform infection in controls, who progressed to a moribund state and death in less than one week. In contrast, all vaccinated animals were asympto-
matic for more than
initial challenge. Th
develop a preventi
primates.

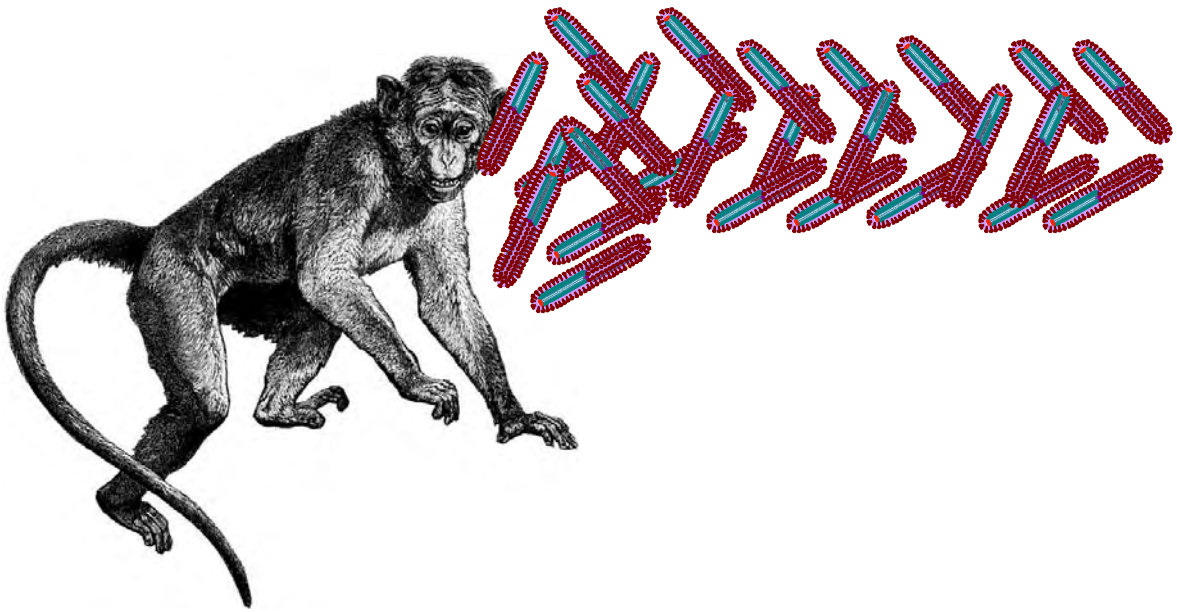
Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates

Nancy J. Sullivan[1], Thomas W. Geisbert[2], Joan B. Geisbert[2], Ling Xu[1], Zhi-yong Yang[1], Mario Roederer[1], Richard A. Koup[1], Peter B. Jahrling[2] & Gary J. Nabel[1]

[1]Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bldg. 40, Room 4502, MSC 3005, 40 Convent Drive, Bethesda, Maryland 20892-3005, USA
[2]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland 21702-5011, USA

Progress on VSV-Based Filovirus Vaccines

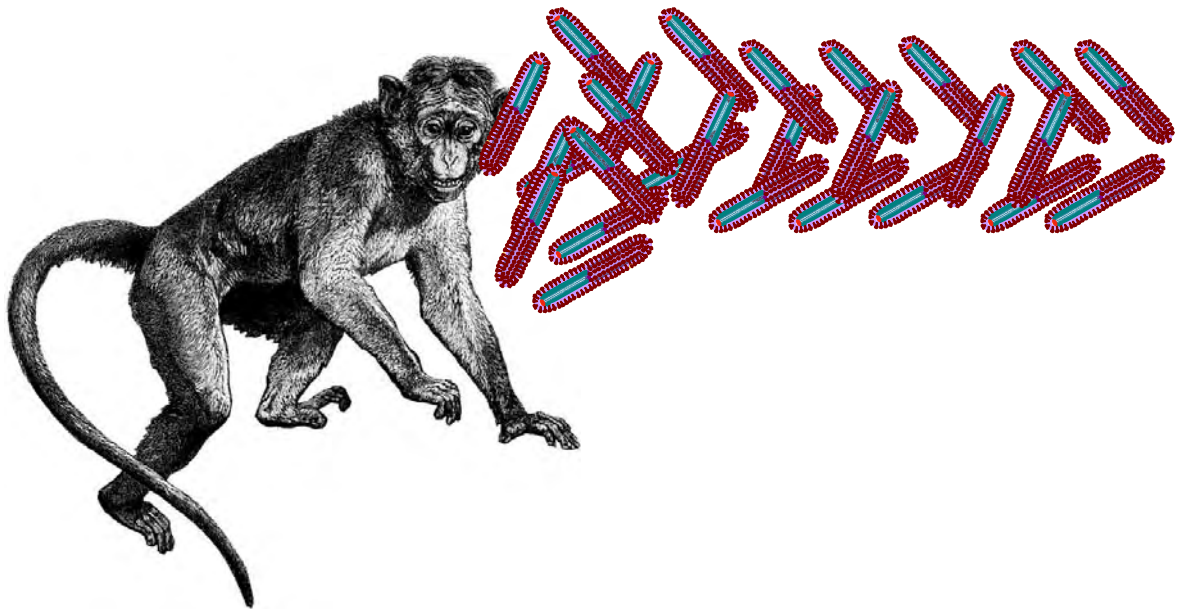
What about an aerosol challenge?



Progress on VSV-Based Filovirus Vaccines

- Single i.m. vaccination with VSV-Ebola-Zaire protects cynomolgus monkeys against an aerosol challenge with Ebola-Zaire

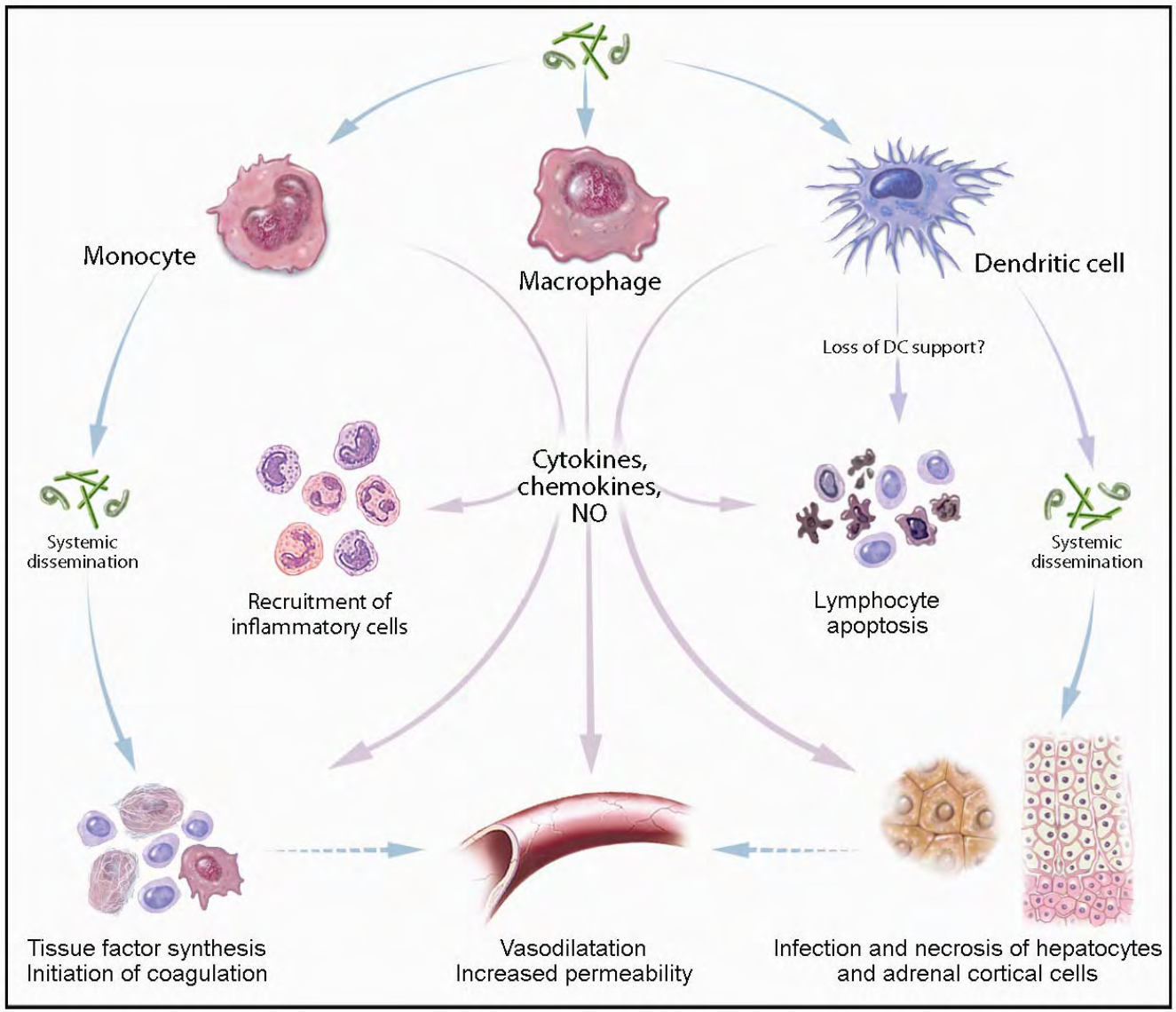
- Single i.m. vaccination with VSV-Marburg (Musoke strain) protects cynomolgus monkeys against an aerosol challenge with Marburg (Musoke strain)



Injection vs. Aerosol

Virus	Route	Number	Mortality	Day of Death
Ebola-Zaire (’95)	i.m. injection	36	100%	6.6 (Mode = 6)
Ebola-Zaire (’95)	aerosol	3	100%	7.3 (7,7,8)
Marburg (Musoke)	i.m. injection	4	100%	9 (9,9,9,9)
Marburg (Musoke)	aerosol	2	100%	11.5 (10,13)

Model of Filovirus Pathogenesis in Primates



Adapted from Fields Virology, 2006

Summary of Clinical Features Filovirus Infection

Feature	Mouse	Guinea Pig	African Green	Cynomolgus Macaque	Rhesus Macaque	Human
Fever	No	Moderate	Yes	Yes	Yes	Yes
Peak viremia	10^8.0-9.0	10^5.0	10^5.5-6.5	10^6.0-7.0	10^6.0-7.0	10^6.5
↑ liver enzymes	Yes	Yes	Yes	Yes	Yes	Yes
Lymphopenia (process of lymphocyte death)	Yes (PCD-like apoptosis)	Yes (?)	Yes (Classical apoptosis)	Yes (Classical apoptosis)	Yes (Classical apoptosis)	Yes (Classical apoptosis)
Neutrophilia	Yes	Yes	Yes	Yes	Yes	Yes
Thrombocytopenia	Modest	Yes	Yes	Yes	Yes	Yes
Macular rash	No	No	No	Yes	Yes	Yes
↑ blood clotting times	No	Yes	Yes	Yes	Yes	Yes
↑ levels of D-dimers	NT	NT	NT	Yes	Yes	Yes
Fibrin deposition	No	Minimal	Moderate	Yes	Yes	Yes
↑ Nitrate levels	NT	NT	NT	Yes	Yes	Yes
In vivo target cells	Mono/Mac, DC?, hepat	Mono/Mac, DC?, hepat	Mono/Mac, DC?, hepat	Mono/Mac, DC, hepat	Mono/Mac, DC, hepat	Mono/Mac, DC, hepat
Increased levels of circulating cytokines	IL-6, TNF-a	NT	NT	IL-6, TNF-a, IFN-a	IL-6, IL-10, TNF-a, IFN-a	IL-6, IL-10, TNF-a, IFN-a

Acknowledgments

Denise Braun

Jeff Brubaker

Doug Reed

Matt Lackemeyer